Phenotyping of 216 accessions of *Miscanthus Sinensis* for saccharification efficiency and cell wall components for QTL mapping

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MSc Major Thesis Report

WAGENINGEN UNIVERSITY
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MSc. Thesis
Bio-based Economy Group
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ABSTRACT

Miscanthus is a perennial plant originating from the tropical regions of Asia while being an ideal biofuel crop due to its nutrient and light use efficiency. As a C_4 crop its rhizomatous nature and high yield with an average of 22.5 tonnes Dry Matter $ha^{-1}yr^{-1}$ is noteworthy mentioning. Marker assisted breeding can help faster breeding for miscanthus and for this reason, in this study, phenotyping of miscanthus was conducted for QTL mapping. Mapping population consisting of from 216 accessions of miscanthus was investigated. Since NIRS technique are known to be high throughput and low cost, In order to phenotype whole mapping population prediction model was utilized which is combination of biochemical and Near-infrared Spectroscopy (NIRS) data for both cell wall composition and saccharification efficiency. Selected 135 plants were phenotyped with regards to cell wall composition and saccharification efficiency traits. R^2 values obtained from the prediction model are as following; NDF%DM (**0.88**), ADF%DM (**0.81**), and CEL%DM (**0.72**), CEL%NDF(**0.60**), HEM%NDF (**0.65**), and HEM%DM (**0.55**), ADL%DM(**0.42**), ADL%NDF(**0.36**), and CelRel%(**0.29**). Transgressive segregation was observed at the mapping population while phenotypically majority of the offspring's were found to be within the range of parents. **Genotypes (G**) among the mapping population were found to be significantly different (<.001) among different traits. Furthermore, by analysing **Year 1** versus **Year 2**, significant difference has also been seen among the population by F value scoring (<.001) for Year factor (Y). Finally, **Genotype by Year interaction (G x Y)** also shows a significant interaction for all cell wall traits listed above (G x Y <.001). This can be translated as that some plant genotypes are affected differently from the year than the others. As has been mentioned, significant differences have confirm that segregation had taken place at the mapping population for all traits investigated. Overall, high heritability values have been observed, the lowest for ndf samples being the 0.747 while 0.762 being the lowest for dry matter (dm) samples. Between year 1 and 2, it can be seen that lignin, and hemicellulose levels were increased and inverse trend was observed for CelRel% and Hem%ndf. In two years, ADL%NDF levels were increased from 7.19 to 10.33; meanwhile, CelRel% (saccharification efficiency related trait) dropped from 40.95 to 24.45 proving recalcitrance impact of lignin. Correlation analysis has shown that **lignin** (ADL.dm) and **cellulose** (Cel.dm) had weak positive correlation as **0.13** . Regarding **lignin** (ADL.dm) and **hemicellulose**(Hem.dm) strong negative correlation has been founded as **-0.46.** As a result, by combining phenotypical data obtained with the genotypic data plenty of candidate QTLs were identified especially for traits such as NDF%DM, CEL%NDF, and HEM%NDF. Especially for the QTLs that are related to the mentioned traits, lots of overlapping had been seen for two years which could be an indication for the validity of the results. QTL results were not included in this report due to time constraints and limitations.

Contents

1-Introduction

1.1 Mischanthus is an ideal biofuel crop due to its high yielding nature, light use efficiency, and its sustainable production way.

Growing world population and increased desired for independence from oil based industry is a recent challenge. Biobased economy offering solutions to this challenge and one of the potential candidate is biofuel crop is Miscanthus. Mischanthus is a perennial plant originating from the tropical regions of Asia, that has a great potential for being used as a sustainable energy crop in temperate climates with its efficient carbon fixation as a C_4 crop (Heaton et al.,2010). There are unique characteristics of Mischanthus which makes it better biomass crop than many other crops, including high yield, broad adaptation, resource use efficiency and sustainability. Mischanthus is a high yielding crop ranging 3-5 meter tall, yield potential may stretch up to 61 tonnes DM (Dry matter) per hectare per year with an average yield of 22.5 tonnes DM $ha^{-1}yr^{-1}$(Byrt et al.,2011). Furthermore, perennial rhizomatous nature of *Miscanthus* is also noteworthy mentioning. Rhizomatous roots are usually underground stem which often sends out roots and shoot from its nodes; furthermore, miscanthus uses C_4 photosynthetic pathway which allow higher nutrient efficiency. The perennial rhizome system allows nutrients to be cycled seasonally between the above and below ground portions of the plant thereby minimizing external additions of fertilizers (Heaton et al.,2004). Furthermore, when harvest is conducted after senescence; in which nutrient translocation has occurred, resultant plant material will have a low mineral content. Therefore, when combustion was performed by using miscanthus, relatively small pollution release could be observed (Lewandoski et al.,1997).

As a perennial C_4 crop, *Mischanthus* use light very efficiently. Also, it has a long growing season which contributes to efficient biomass build. Furthermore, when comparisons were made with regards to relative dry matter production of *Mischanthus* compared to another dominant and proven biomass crop , *switchgrass,* it was concluded that *Mischanthus* has more less similar interception and conversion of solar radiation to biomass leading a very high dry matter production(Heaton et al.,2008). In addition to its efficient light use, it also has a high efficiency of water use, approximately it requires between 100 and 300 l of water to produce 1 kg biomass(Heaton et al.,2010). In case of an annual maize or sorghum crop, this value is around 300 l kg^{-1}(Howell et al.,1998).

Perennial crop such as *Mischanthus* is associated with good environmental performance and ecosystem health. Soil tillage is not disturbed below and above ground while biomass accumulates and the soil is protected against wind/ water erosion. Also, perennial crops increases the quantity and diversity of mineral nutrients available at the soil thereby contributing to environmental sustainability.

1.2 Cell wall constituents and saccharification efficiency are important parameters for a viable bio-fuel crop.

Lignocellulose is the most abundant biomass on earth (Xu et al.,2012) and cell wall corresponds to a large segment of plant biomass especially at the lignocellulosic plants. Cell wall mostly consists of from the two principal carbohydrate polymers namely cellulose and hemicellulose. Cellulose being the major polysaccharide accounting for 28-30% of dry matter and it is composed of high molecular weight linear polymer composed of B-

1, 4 –glucans (Ning et al, 2012). Lignin also took apart in the composition of cell wall during evolutionary adaptations to resist enzymatic breakdown by invaders. Removing lignin (for instance by pressure and high temperature or by acids) has being implemented at commercial level; however, lignin's recalcitrance overall increases processing costs. Having low lignin and high hemicellulose/ cellulose levels would be desired trait for biofuel crop. On the other hand, even though decreasing lignin content would be good for saccharification, thermochemical conversion applications would prefer an increased lignin content to improve caloric value and energy density (Alison et al.,2011).

In order to have a better conversion with lower cost of plant biomass into biofuel, current research trend focuses on plant cell walls composition and its conversion easiness of the biomass into energy. Based on many researches (Grabber, 2005; Lygin et al., 2011; Vanholme et al.,2012), it is shown that recalcitrance is directly correlated with lignin levels. However, lignin is not the only cell wall component that influences enzymatic saccharification (Torres et al.,2014).It has been shown that distribution of sugar and/or non sugar branching substituents in the main chain of the polysaccharides also contributes to recalcitrance (De Souze et al., 2015). Breeding may help us to have optimised cell wall composition by using natural genetic variation.

Another important trait beside the cell wall composition is Saccharification Efficiency (SE) which is related to release of neutral sugars such as glucose and xylose from the cell wall . Just as natural variation in cell wall having an impact on SE, Lignin's recalcitrance also affect the release of sugars by preventing access of enzymes to cellulose during hydrolysis (Jung et al.,1986)(Himmels et al.,2007). It is known that arabinoxylan and xyloglucan contributes positively to hydrolysis.(De Souza et al.,2015). Furthermore, high complexity of the cell wall components with the presence of vascular bundles and their density are important factors determining sugar release from the cell wall(Himmels et al.,2007). Therefore, SE is one of the important trait associated with final yield of bioethanol production. In theory, we could have a plant having high cellulose and hemicellulose levels but low saccharification efficiency which could mean that alternative factors such as cell wall architecture, crystilinization,etc... could influence the release of fermentable sugars from the cell wall.

Another interesting correlation observed is related to cell wall composition. When cellulose and lignin levels were measured from three different groups (respectively *M.x giganteus, M. sacchariflorus, M.sinensis)* decreased cellulose content was measured in an orderly fashion (from left to right) and respectively 47.09%, 42.94%, and 42.50% ratios were obtained (in NDF). Similar pattern was observed for lignin levels as well such that lignin levels for *M.x giganteus* was calculated as 11.76% and were followed by 10.15%, and 9.07%. However, hemicellulose levels did not follow the same trend as it was for both cellulose and lignin levels but had an inverse correlation. More clearly, when cellulose and lignin levels were increased, hemicellulose levels were decreased(Alison et al., 2011). Therefore, as can be seen from the above case as well, manipulation of cell wall composition could have direct and also indirect impact on the quality of the plant material thereby biofuel processing.

1.3 QTL Mapping enables faster breeding programs by discovering location of quantitative trait on chromosomes.

The process of constructing a linkage maps and conducting QTL analysis to identify genomic regions associated with trait is known as QTL mapping(Collard et al.,2005). Finding genome regions that contain genes associated with saccharification efficiency, and cell wall components is one of the goals of this thesis. The fundamental principle of QTL mapping is that genes and markers segregate via chromosome recombination (called crossing over) during meiosis (in our case, sexual production of mapping population from 2 *Mischanthus Sinesis* Parents) thereby allowing their analyses in the progeny(Collar et al.,2005). Parents selected for this mapping population also should be phenotypical different for one or more traits (for this research contrasting cell wall composition). *Mischanthus Sinesis* is a diploid plant (2n=2x=38) and as an allogomous plant, natural variation in cell wall composition is quite high and progeny from the cross expected to have a unique genotype resembling parents due to recombination (Hodkinson et al.,2002). Furthermore, transgressive segregation can also be potentially seen at the progeny which will be explained by the comparison of phenotypical data at the results and discussion section. Additionally, by identification of QTL regions using this mapping population, new varieties could be developed with a high biomass quality for biofuel production. Genes or markers close or tightly linked together will be transmitted together from parent to progeny more frequently than genes or markers that are located further apart. Mapping populations for preliminary genetic mapping ranges between 50 to 250 (Mohan et al.,1997, and regarding this research, population size is 216. Furthermore, mapping populations must be phenotypically evaluated which had been done by the combination of both biochemical lab analysis and NIRs scanning. In our case, prediction model (referring Figure 3) used for assessing phenotypical characteristics of the whole population (further explanation given at material and methods section). By identifying QTL regions that are important for traits such as Saccharification Efficiency (SE), and cell wall composition, future breeding projects can make use of the research conducted and cultivars having high SE with a desired cell wall composition can be obtained based on breeding target. In future studies, one or a few desired QTLs can be easily introgressed into elite lines through Marker assisted backcrossing. Furthermore, perennial crop like miscanthus would take 3 years to complete its growth and therefore selecting at the seedling stage would fasten the of breeding instead of waiting 3 years.

Objectives of the thesis

Main goal of this thesis is constructing a QTL mapping by using a prediction model based on 143 different accessions of *Mischanthus Sinesis* with regards to cell wall composition (such as lignin, hemicellulose, and cellulose) and saccharification efficiency. Therefore, phenotyping part of the thesis will utilize both biochemical analysis and Near infrared light scanning (NIRS) prediction. Mapping population used consist of from a cross between 2 *Mischanthus Sinesis parents*. Ultimate goal of the thesis is helping breeding projects by the discovery of QTLs which would be used in marker assisted selection in future as a part of bio-based economy.

- All 1296 samples were scanned by NIRS
- 143 samples (100 samples for construction of calibration model and 43 samples were used for cross validation of the constructed model) were selected to represent most of the spectral variation.
- Analyse 143 samples biochemically for cellulose, hemicellulose, lignin, and saccharification efficiency
- Predicting phenotypical composition of whole mapping population (Year 1+Year 2) by using prediction model.
- Analyse the dataset for genotypic variation in biomass quality.
- Identification of candidate QTL regions correlated with saccharification efficiency, and cell wall composition.
- By discovering candidate QTL regions correlated with saccharification efficiency and cell components, the project aims to help for marker assisted breeding for biofuel production.

2-Materials and Methods

1. Plant Material

Total field plantation consisted of from 648 plants in 3 blocks (including 2 parents+646 offspring's) in Wageningen/Netherlands. Plants were planted as a completely randomized block design having a 3 replicates per genotype. Harvest was conducted after complete senescence was achieved. Leaves were separated from the stem and the stem samples were chopped down to less than 1 cm pieces with a chopping machine. Plant materials were grinded and stored in plastic tubes. For each accessions, 5 replicates were made (2* for DMC, ADF, ADL, and 3* for Saccharification efficiency).

The cross between parent 1 and 2 resulted with the mapping population obtained. Unfortunately, during field experimentation, **parent 2** was dead and also for **the first year**, **parent 1** was not grown fully therefore sample biomass could not have been taken. Due to the constraints mentioned earlier, phenotypical data for parents of the mapping population is not available for year 1. However, second years data includes sample from parent 1 so deeper analysis can be conducted for second year

2. Biochemical Analysis

- ### DMC,NDF, ADF, and ADL

Dry Matter Content (DMC) obtains biomass weight without moisture

ANKOM F57 bags were pre-weighed before addition of samples. 0.50 to 0.49 grams of samples were added to each bag and then bags were sealed. Bags were left overnight at 103 degree. Following day, samples were put in the a sealed plastic bags including silica bags to prevent moisture infiltration. Samples were cooled down for an hour and then the weight of fully dried samples were measured. Total number of selected plants was 143. For phenotypical analysis; namely DMC,ADF, and ADL, 2 replicates were made. Regarding for Saccharification Efficiency experiment, 3 replicates were made for each of these 143 samples. However, some of the grinded samples were not enough with regards to biomass; therefore, some of the samples were excluded from the experiment. Furthermore, for each 23 samples, 1 control sample was produced. Overall, 715 samples were produced for all of the phenotypical experimentations; moreover, 30 additional control samples were added to the experimentation (total number omits missing samples due to available low biomass). In order to calculate the DMC the following formula was used;

Formula 1

$$DMC\ (\%) = \frac{Total\ Dry\ Weight(g) - Bag\ Weight - (Correction\ factor)\ 0.0025}{Sample\ Weight}$$

Biochemical Analysis helps identifying Hemicellulose, Cellulose and Lignin amount

Following table 1 was constructed for measuring different cell wall components. NDF washes away all non-cell wall components. ADF washes away hemicellulose while ADL removes all cellulose content.

Table 2: Cell wall components after treatments of respectively NDF, ADF, and lastly ADL

Buffer	Remaining Cell Wall Content		
NDF	Cellulose	Hemicellulose	Lignin
ADF	Cellulose	Lignin	
ADL	Lignin		

Table 3: Formulas used for calculation of Hemicellulose, Cellulose, and lignin

	Cell wall components (Hemicellulose,Cellulose,Lignin as %dm)
$\mathrm{NDF\%dm} - \mathrm{ADF\%dm}$	Hemicellulose % dm
$\mathrm{ADF\%dm} - \mathrm{ADL\%dm}$	Cellulose % dm
$\mathrm{ADL\%dm}$	Ligning % dm

Table 2 summarizes the overall biochemical extraction methods used and formulas used to obtaining extracted different cell wall components related to biomass. For ADL experimentation, samples that are treated with ADF were used therefore hemicellulose was pre-washed at this experiment already. By utilizing the table 2, Cel%ndf, Hem%ndf, and Lig%ndf can be calculated.

$$Cel\%ndf = \frac{ADF\%DM - ADL\%DM}{NDF\%DM} \times 100$$

$$Hem\%ndf = \frac{NDF\%DM - ADF\%DM}{NDF\%DM} \times 100 \qquad Lig\%ndf = \frac{ADL\%DM}{NDF\%DM} \times 100$$

Neutral Detergent Fibre (NDF) helps eliminating all non-cell wall components including starch,lipids, and proteins.

NDF treatment consists of from sodium dedocyl sulfate, ethylendiamine-tetraacetic acid disodium salt dihydrate, sodium tetraborate decahydrate, sodium phosphate dibasic, and triethylene glycol. The use of NDF aims removing all of the non-cell wall components. Heat-stable bacterial alpha amylase and sodium sulphate(Na_2SO_3) were added for incubation for 100°C while using NDF program from Ankom machinery. After an incubation about 75 minutes, further steps were conducted (twice with alpha amylase solution and once with acetone 10 min.). After keeping samples in acetone for 10 minutes, samples were put in overnight at 103°C. Following day, samples were cooled and weighed. Furthermore, by using the below formula calculations were made. NDF treatment aims removing all non-cell wall components.

Formula 2

$$NDF(\%) = \frac{\text{Total weight after NDF treatment} - \text{Original bag weight} + 0.0048\,*}{\text{Dried sample weight}} \times 100\%$$

*0.0048 correction factor calculated by weighing empty bags and average has been taken(before and after NDF treatment) as a correction factor.

The NDF data used for this experiment was conducted in 2014.

Acid Detergent Fibre (ADF) removes all hemicellulose from cell wall

ADF buffer solution consists of from 20 g cetyl trimethylammonium bromide (CATB) which is added to 1 L demi-water with 26.6 ml of 98% sulfuric acid (H_2SO_4)). Then Ankom machine's ADF program was in which temperature was set up to 100°C for 60 min. After rinsing process, samples were put in acetone for 10 minutes and then were dried overnight at 103°C. Following day, samples were cooled and weighed. ADF buffer washes away hemicellulose.

Formula 3

$$ADF(\%) = \frac{\text{Total weight after ADF treatment} - \text{Original bag weight} + 0.00031\,*}{\text{Dried sample weight}} \times 100\%$$

*0.00031 is the correction factor calculated by empty bags measured (before and after ADF treatment).

Correction factors expected to be a negative (calculated average value for **empty bag without treatment** - **treated empty**(*treatment means ADF,ADL,NDF, or DMC)=**correction value**) value in this experiment based on the assumption that the treatments (such as ADL,ADF, and NDF) that applied could have cause biomass lose at the filter bags or also eliminating moisture in case for DMC. However, for **formulas 2,** and **3**, the correction factor is calculated as a positive number which means that the biomass of empty filter bags had increased after ADF and NDF treatments. Plausible explanation for this situation would be that during the experimentation, empty bags were put in ANKOM with other samples(22 samples+1 control+1empty bag); furthermore, during ADF, and NDF washing, some of the washed away samples may have been stack at empty bags thereby increasing the weighed biomass after these treatments. Opposing to ADF, NDF cases, negative correction factor calculation was observed for ADL and DMC. With regards to DMC, elimination of moisture at bags by overnight drying can cause negative correction factor. As has been said earlier, during ADL treatment (which is quite harsh acid treatment 72% H_2SO_4) biomass of the empty bag would expected to be lessen thereby having an expected negative value in the end.

Acid Detergent Lignin (ADL) washes away cellulose

After conducting ADF experimentation, ADL experiment has been performed on the same samples. For this experimentation, 98% H_2SO_4 was diluted to 72% H_2SO_4. For every litre, 713 Ml of 98% H_2SO_4 was added to 500ml of water. For 24 samples, 700 ml of 72% H_2SO_4 was used. Samples were immersed with the acid at the shaker (90rpm) for 3 hours. Then, samples were rinsed in water (10 washing steps). Lastly samples were put in acetone for 10 minutes, and then dried overnight 103°C and weighed. ADL content was measured by the following formula;

Formula 4

$$ADL(\%) = \frac{Total\ weight\ after\ ADL\ treatment - Original\ bag\ weight - 0.00467*}{Dry\ sample\ weight}$$

*0.00467 is the correction factor calculated as the average of blank bag weights (before and after ADL treatment)

Saccharification Efficiency (SE) is an important biofuel trait associated with release of monosaccharides such as Glucose and Xylose from polysaccharide cell wall

SE experiment consisted of from 3 major parts. 1^{st} step is washing away soluble sugars (monosaccharides) and starch. For this purpose, samples were washed using ANKOM. Customized program was used in which 13 minutes acid extraction option selected and the temperature was set up to 50^0C including 3 washing steps. Temperature was kept low at 50^0C to prevent full breakage of bonds which would eliminate all differences among different genotypes. Just as conducted for ADF,ADL, and NDF, parameters used at SE treatments were way milder than the industrial treatments which would give us a better sense to see phenotypical differences among different cultivar. Strong treatment would eliminate most of the differences among the mapping population. Therefore, it would not be easy to see which genotypes segregating for the traits investigated. At each washing step, 4 ml of thermostable alpha amylase was added. Alpha amylase would help washing away starch. Later on, 15 ml of 2%NaOH was added for 2 hours of incubation. Alkaline solution would wash away chloroplast, proteins, and monosaccharide sugars as non-cell wall compositions. Washing with alkaline treatment would target ester bonds between cellulose, hemicellulose and lignin(Fry.,1986) . After alkaline treatment, samples were washed twice (40 ml) demi-water and incubated for 5 minutes each time (50 ^{0}C, 160 rpm). After rinsing with demi-water twice, tubes were filled with 15 ml of 0.1 M sodium citrate buffer with the same shaking settings. Second step includes putting samples in 50 ml blue capped tubes with a sodium citrate buffer(44 ml) which has sodium benzoate (which would prevent microbe growth). To this buffer, 6.8 ul of xylase enzyme and 0.3 ml of Accellerase 1500 (Finland) enzyme was added. Later on, prepared samples were put in incubator for 48 hours (50 ^{0}C, 160 rpm). Sodium citrate buffer works by providing stable environment (stable pH) which will provide appropriate environment for enzymes to work. Accellerase 1500 (Finland) enzyme functions as a cocktail of enzymes digesting cell wall components. Overall, Accellerase ensures conversion of lignocellulosic cell wall into fermentable sugars such as glucose. It contains high levels of beta-glucosidase activity to ensure almost conversion of cellobiose to glucose(Accellerase,Dupont Trd). Furthermore, xylase enzyme targets polysaccharides and convert them into xylose mono sugars. After incubation for 48 hours (50 ^{0}C, 160 rpm), 2ml of sample were taken from each 44 ml tubes with a syringe. Samples were filtered with 0.45 µm. In order to stop activity of enzymes, filtered samples were incubated for 5.5 minutes in thermomixer at 99^{0}C with 300 rpm. After all the enzyme activities are terminated, 20 ul of sample were taken from the two ml Eppendorf tube and this samples was transferred into 980 ul miliqwater(MQ water) in a dionex tube. Further analysis was conducted with dionex tubes while using HPLC (High pressure liquid chromatography). Release of glucose and xylose sugar was captured by HPLC and based on the standard curve (include known concentration, and known amount of released sugars; namely, glucose and xylose) glucose and xylose concentration were calculated. Since from each sample, 20 ul of sample has been taken and transferred into 980 ul of MQ, dilution factor is 50.

Released Glucose content

Formula 5

$$\begin{aligned}&Total\ Produced/released\ Glucose(mg)\\&\qquad = Concentration\ Glucose\left(\frac{mg}{ml}\right) \times Dilution\ factor(50) \times Total\ volume(ml)\end{aligned}$$

Formula 6

$$GluRel\%dm = \frac{\frac{Total\ Produced/released\ Glucose(mg)}{1000}}{Weight\ of\ sample(g) \times DMC\%}$$

Formula 7

$$GlucoseRel(\%) = \frac{Total\ Produced/released\ Glucose(mg)}{Total\ Glucose\ from\ the\ NDF\ cell\ wall\ *}$$

*Total Glucose from the cell wall is calculated as following;

$$Sample\ weight(g) \times DMC\% \times NDF\% \times Glucose\ \%NDF \times 1000$$

Formula 7 shows ratio of released glucose from the cell wall. However, Formula 6 shows the ratio between total produced/released Glucose comparison to the whole dry matter of sample including non-cell walled components.

Both formulas 6 and 7 can help us to see how much of Glucose sugar has been produced/released per gram of a sample from the whole as DMC (cell wall +non cell wall components from samples, altogether), and also just as solely from the cell wall (hemicellulose,cellulose,and lignin).

Released Xylose content

Formula 8

$$Total\ Produced/released\ Xylose(mg) = ConcentrationXylose\left(\frac{mg}{ml}\right) \times Dilution\ factor(50) \times Total\ volume(ml)$$

Formula 9

$$GluRel\%dm = \frac{\frac{Total\ Produced/released\ Xylose(mg)}{1000}}{Weight\ of\ sample(g) \times DMC\%}$$

Formula 10

$$GlucoseRel(\%) = \frac{Total\ Produced/released\ Xylose(mg)}{Total\ Glucose\ from\ the\ NDF\ cell\ wall *}$$

*Total Glucose from the cell wall is calculated as following;

$$Sample\ weight(g) \times DMC\% \times NDF\% \times Xylose\ \%NDF \times 1000$$

For both Glucose and Xylose sugar concentrations (mg/ml) were calculated based on the standard curve obtained.

Following common formula used for calculations;

Formula 11

*Peak area obtained from the dionex analysis(HPLC)× Slope of the standard curve+ intercept. Furthermore, correlation coefficient has been checked for more accurate results.

Near-infrared spectroscopy (NIRs) is high through put method help phenotyping large populations

In order to phenotype all population which consist of from 648 plots, preferably biochemical analysis should have been conducted for target traits such as saccharification efficiency, and cell wall composition. Due to time constraints and economical costs of biochemically analysing such a large population, alternative method had been utilized. Therefore, NIRs was used for fully phenotyping the Mischanthus population. The basic idea behind NIRs analysis consists of from **i**- construction of calibration model by analysing 100 samples both biochemically and also by NIRs scanning, **ii-** validation of calibration model by selected samples which consist of from 43 samples and upgrading the model by further biochemically analysing the outliers from the validation, **iii-** predicting phenotypical data of the whole population by using the prediction model for both year 1 and 2 (1108 samples total).

Near-infrared spectroscopy (NIRS) is a fast, practical, and non-destructive technology. Firstly, samples were mixed thoroughly and added to a small cup with a quartz glass (50 mm diameter,10mm depth) and scanned quickly. Spectral data has been obtained based absorbance of samples; furthermore, the absorbance response to chemical composition (in our case saccharification efficiency, cell wall composition) is non-linear (Xu et al.,2013). Therefore, principal component analysis (PCA) and partial least squares (PLS) was used to obtain prediction model(Xu et al.,2013).The software 'Mosaic Solo (NIRS DS2500) combined NIRs data and by using Software program WinISI III (Version 1.50, Infrasoft International LLC, PA, USA), location of samples at the spectral hypersphere of the calibration was found. Two standards were the basis of selection which are Global H (GH) and Neighbourhood H (NH). These standards shows how different are the samples from the average. GH is the distance of every sample from the central of the spectral hypersphere. Limit value for GH number is 3. NH has used for controlling the closeness of neighbouring samples within the calibration set and it is distance to closest neighbouring sample. For NH, the limit was set as 0.60. Overall, values higher than for GH,3 and for NH,0.6 considered to be an outlier. By using NH model; which shows distance to the closest neighbour, one sample may represent many of the neighbouring samples thereby fewer samples may have been required which could save lots of time, effort, and economical costs (for biochemical analysis). Another important point to make is that while we were choosing 143 samples, selection was based on the variation that samples had similar to the idea of NH. Instead of closely variated samples, selection was based on large variation among samples which could have greater power predicting larger variation among the mapping population.

One of the advantages of using NIRS is its Intensity Correction (ERC standard). ERC provides for each measurements a true spectroscopic reference which is important to achieve high quality spectrum on instrument and secure transferability between instruments. ERC would help to correct possible differences caused by external factors such as glass impurities. Applied correction to every spectrum to make each spectrum appear as if taken with a 100 % reflectance (NIRs manual , 2006)

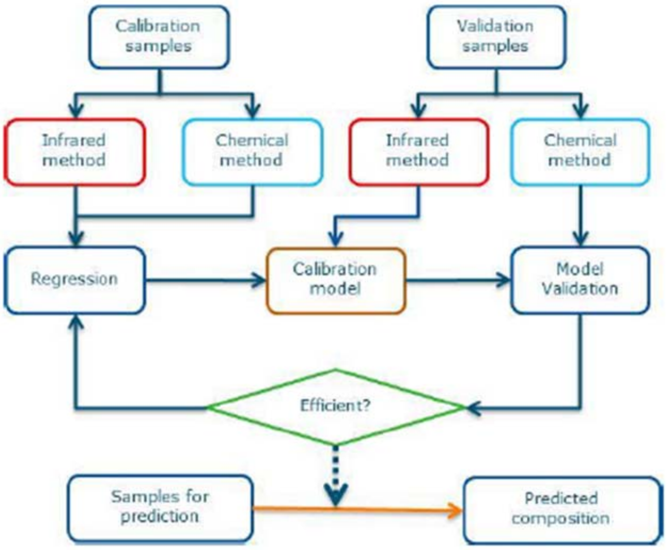


Figure 1.Constructing quantitative prediction model using infrared techniques (Xu et al., 2013)

Initially above prediction model was used; however, in order to strengthen the power of prediction later on cross validation prediction was used which has been shown below.

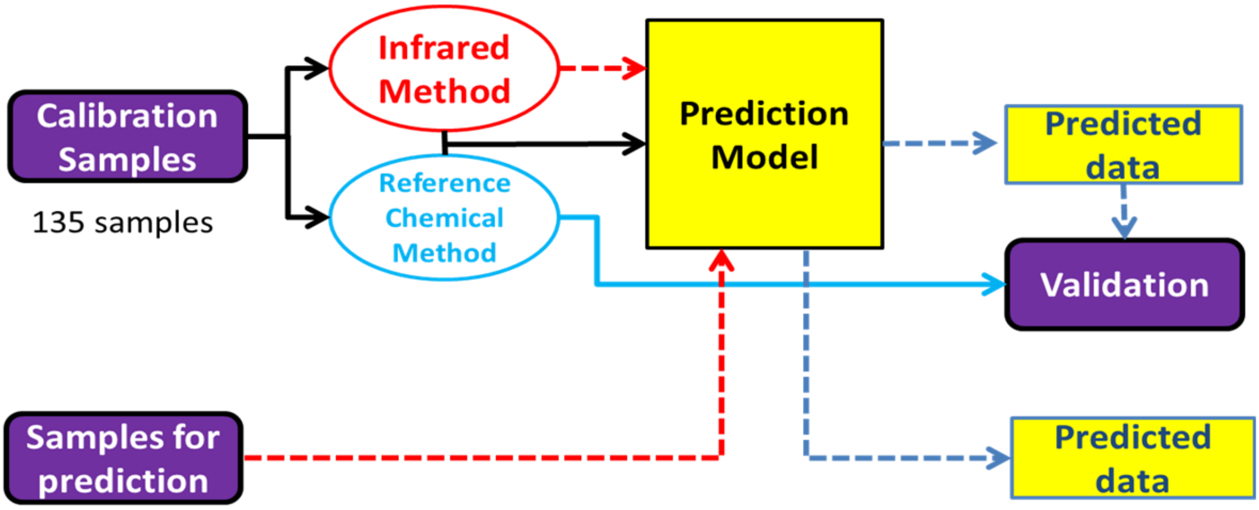
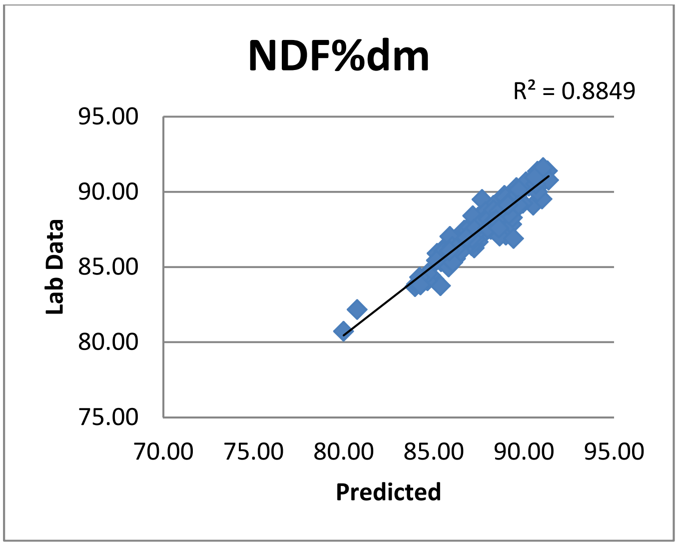
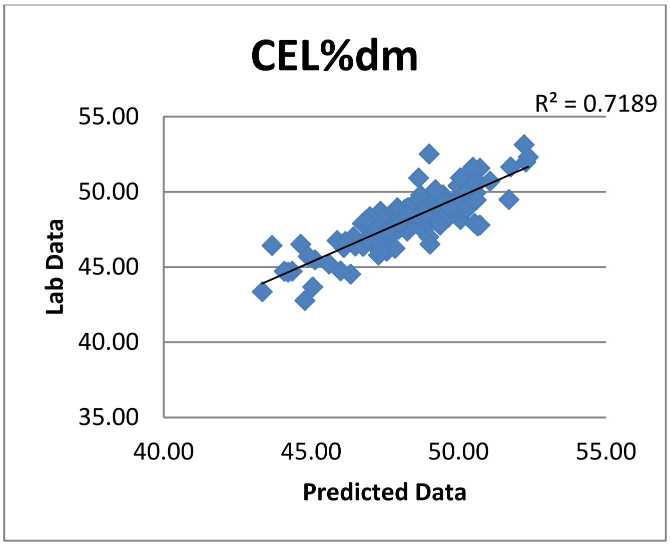


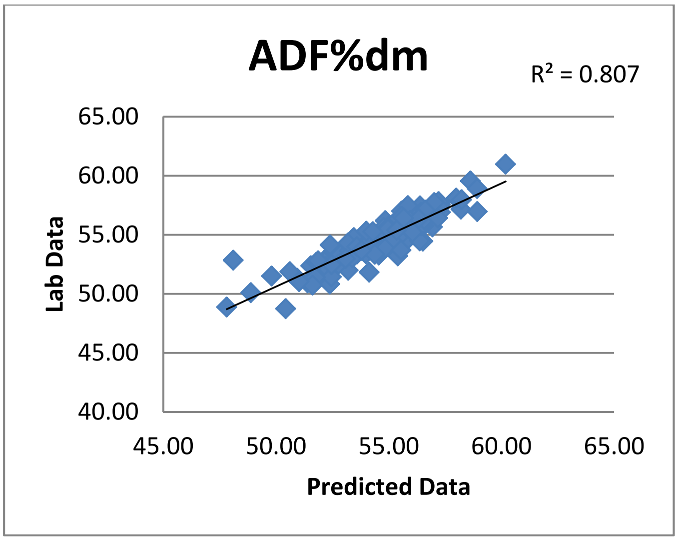
Figure 2. Prediction model used including cross validation of the predicted data.

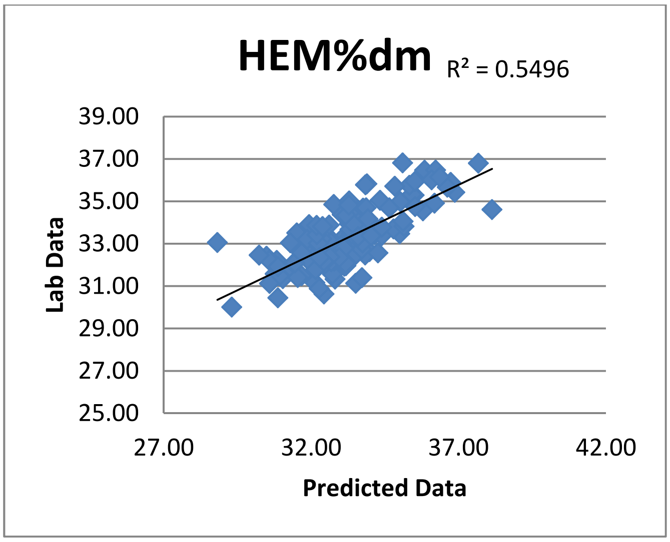
3-Results and Discussion

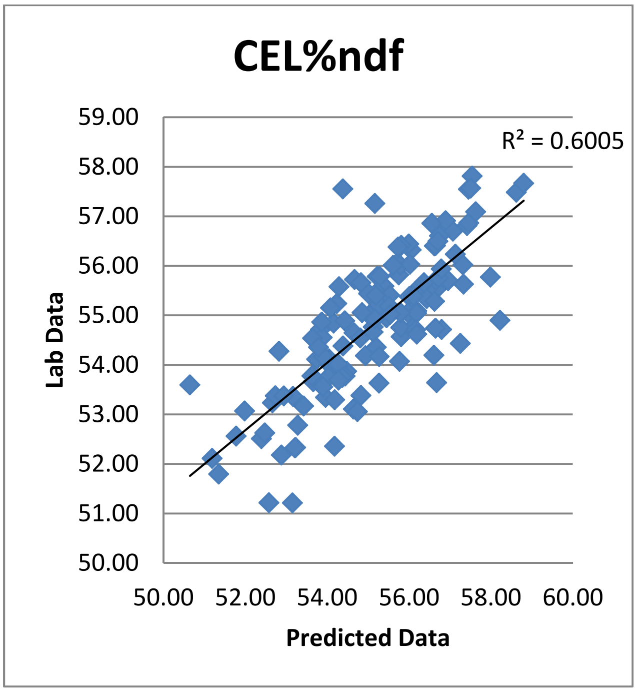
1.1 High through put method such as NIRs prediction model which can save both time and money for phenotyping has been validated for different traits

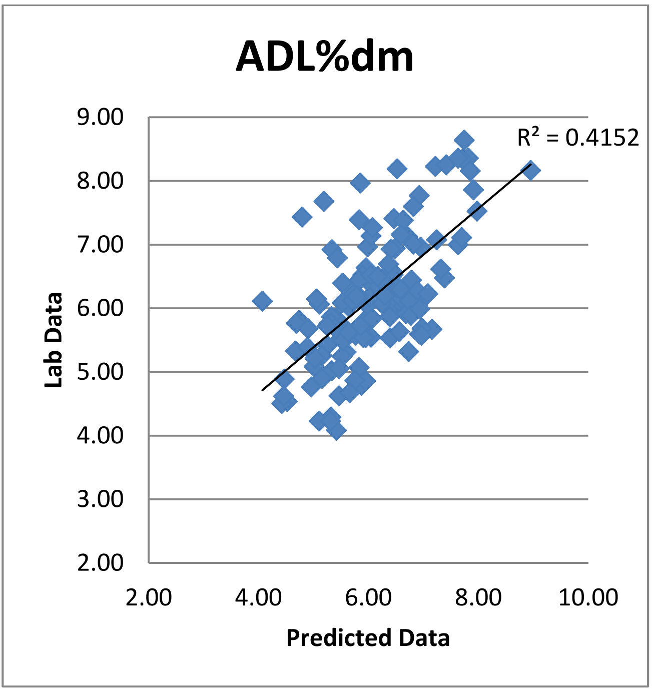


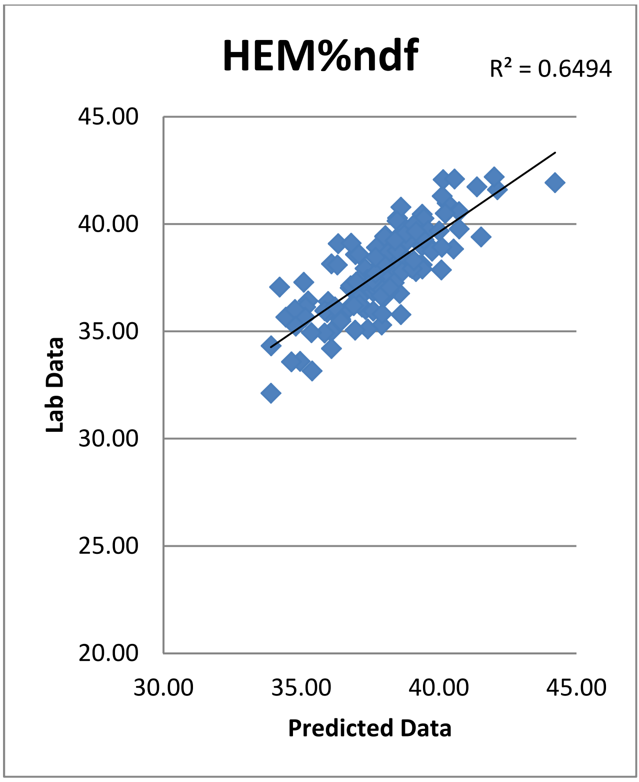


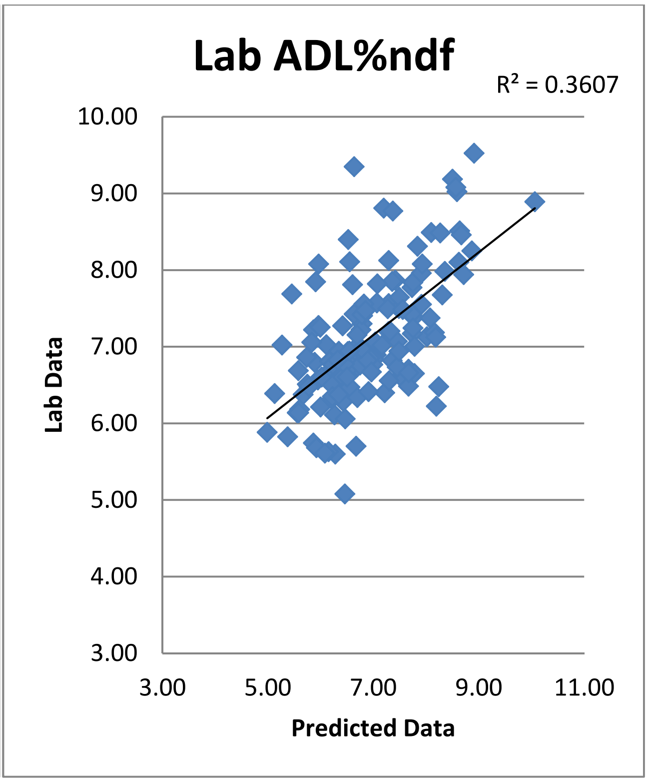





CEL%ndf
R² = 0.6005
Lab Data
59.00
58.00
57.00
56.00
55.00
54.00
53.00
52.00
51.00
50.00
50.00
52.00
54.00
56.00
58.00
60.00
Predicted Data


ADL%dm
R² = 0.4152
Lab Data
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
2.00
4.00
6.00
8.00
10.00
Predicted Data


HEM%ndf
R² = 0.6494
Lab Data
45.00
40.00
35.00
30.00
25.00
20.00
30.00
35.00
40.00
45.00
Predicted Data


Lab ADL%ndf
R² = 0.3607
Lab Data
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
3.00
5.00
7.00
9.00
11.00
Predicted Data

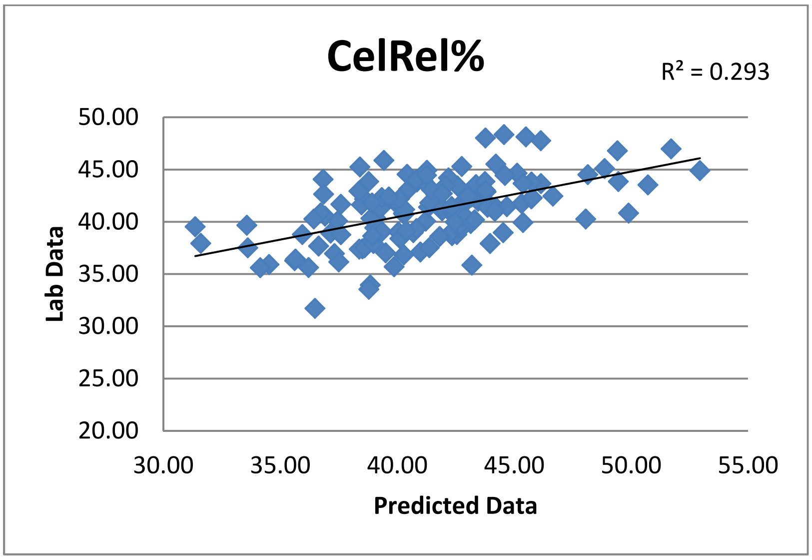


Figure 3.Correlation between the Lab Data versus Predicted Data with regards to different cell wall components for Year 1

For all analyzed traits, a comparison was made between the lab and NIRS-predicted data in order to validate the prediction equations (Figure 2). Large variation was observed at the calculated R-squared values for the mentioned traits. With regards to traits such as NDF%DM (**0.88**), ADF%DM (**0.81**), and CEL%DM (**0.72**), calculated R-squared numbers were among the highest in the whole group which would give stronger power of prediction. Furthermore, CEL%NDF(**0.6001**), HEM%NDF (**0.649**), and HEM%DM (**0.549**) traits scored lower R-squared values compared to the first group mentioned. However, HEM%NDF which just includes cell wall components scored higher than the HEM%DM. As has been shown at the material and methods section, Hem%dm and Hem%ndf refer to the same component but just expressed as percentage of a different weight starting material. Lastly, ADL%DM(**0.4152**), ADL%NDF(**0.3607**), and CelRel%(**0.293**) were found as the lowest R-squared values compared to all 9 traits and further analysis will be elaborated causing low R-squared values.

First of all, low R-squared value could mean that the model used for predicting different traits cannot perform precise prediction for the selected genotypes. There are different factors can contribute to the low R-squared value such as, large experimental errors, too low number of samples used for calibration of the prediction model. For instance, the trait chosen to be predicted by the prediction model could be hard to measure. CelRel% which scored the lowest has a quite laborious and detailed lab protocol to measure. Therefore, it is easier to introduce measuring errors to the observation. Hydrolysis reaction at saccharification experiments are by nature hard to control and this would be the main challenge contributing to the low prediction power. Also, amount of glucose and xylose being measured are highly diluted (50 times). Even a small mistake during lab analysis can introduce large variation in measurements. Overall, when looked from NDF (less complex) treatments toward to CelRel% (highly complex), it could be seen that the more complex the experimentation is, the more variation observed from the results. Therefore, it could be concluded that for complex traits (which are hard to quantify) such as CelRel%, different prediction model can be used for more precise estimation; meanwhile, alternative preferably less complex lab techniques can be investigated and used in order to introduce less error thereby strengthening prediction power of the model. Overall, number of replicates could be increased and more samples could be included for calibration in order to have larger R-squared values for the prediction model.

2.1Transgressive segregation could be an indication for contrasting parental characteristics in Year 1 mapping population.

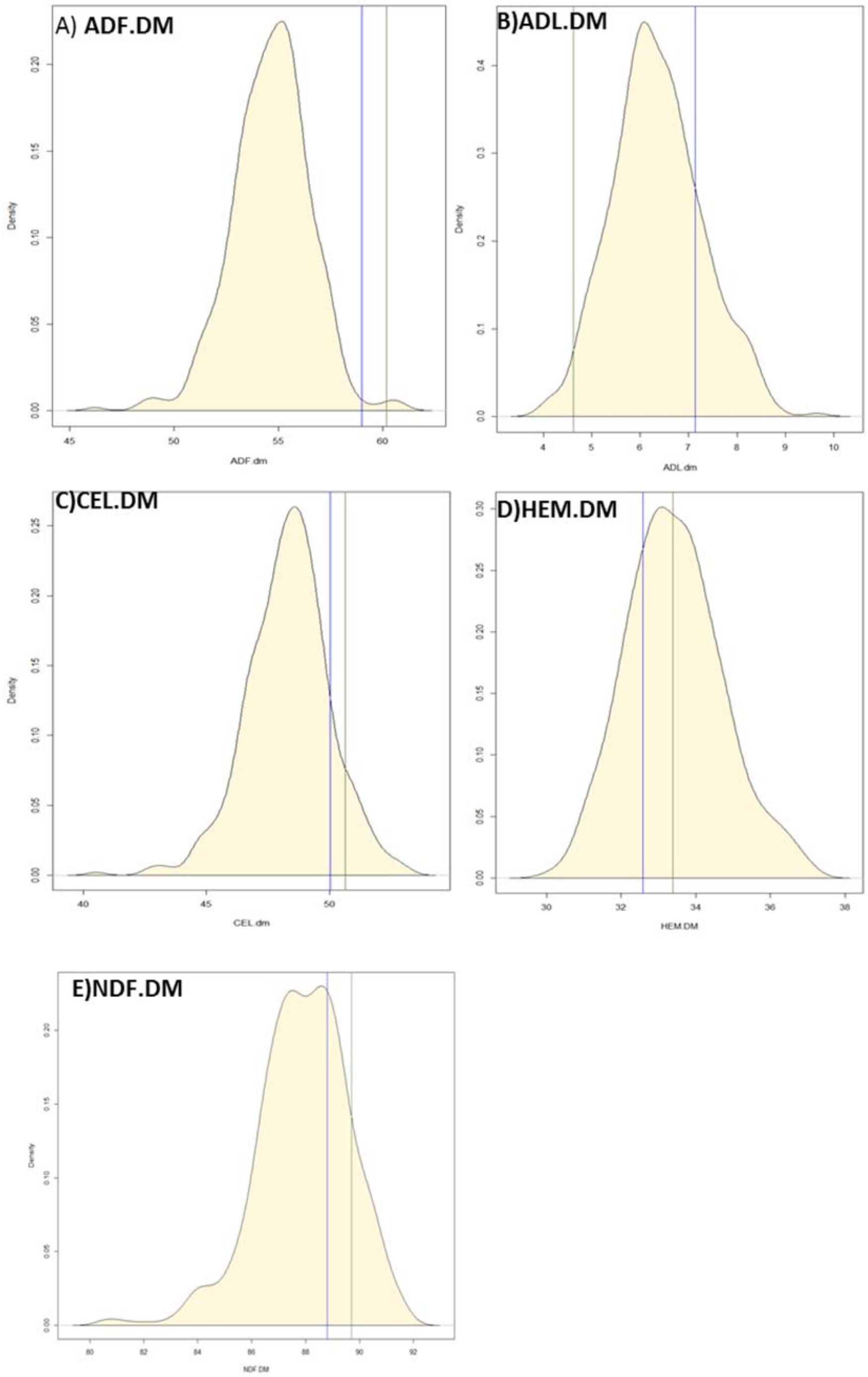


Figure 3a. Density curve for **ADF, ADL, CEL, HEM, and NDF** dry matter **(dm)** contents where blue line being Grandparent 2 of Parent 2 and Green being Grandparent 3 of Parent 2.

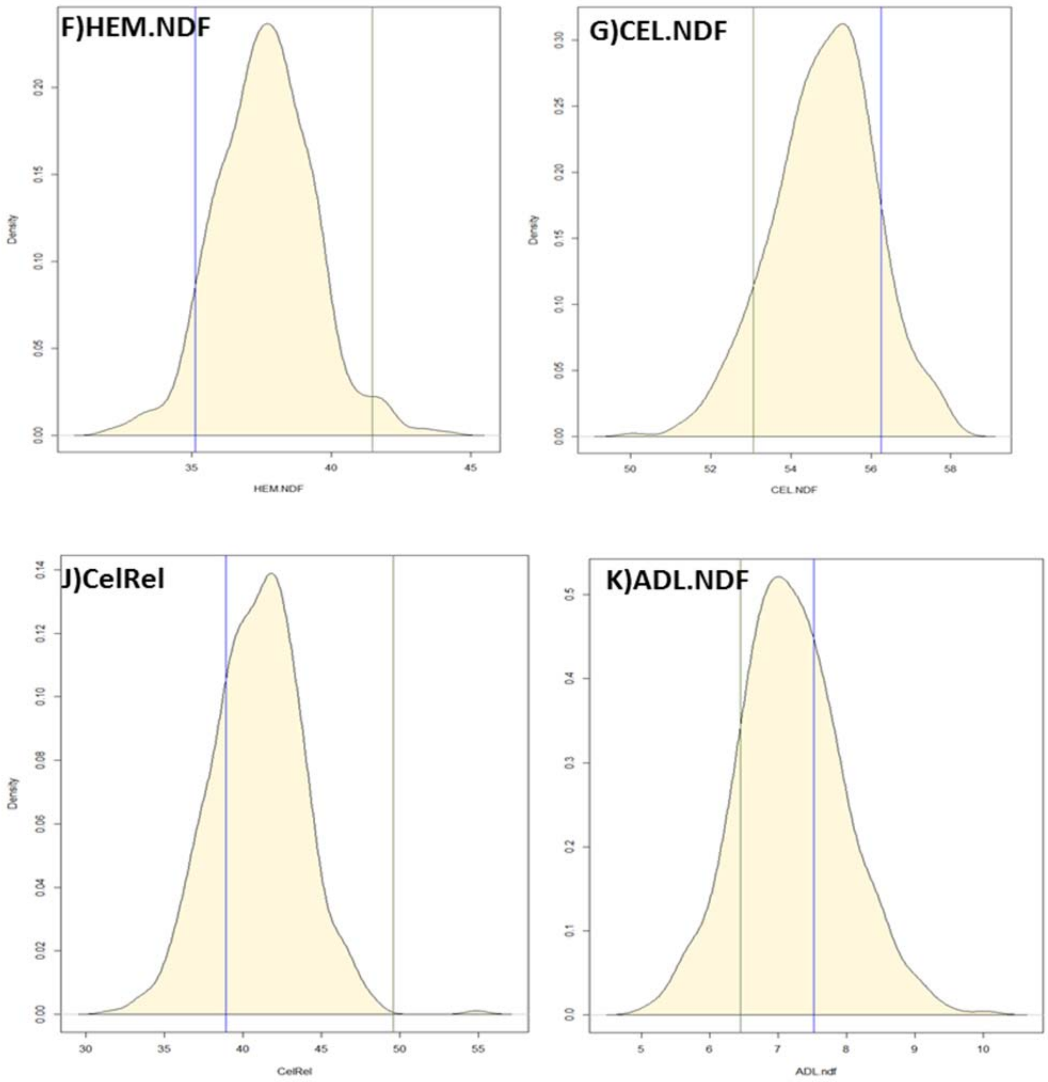


Figure 3b. Density curve versus different cell wall traits for year 1, while blue line being Grandparent 2 of Parent 2 and green being Grandparent 3 of Parent 2 for Hemicellulose, cellulose, lignin and CelRel traits for year 1.

In order to observe segregation among mapping population and conducting comparison analysis with the parents, phenotypical data from grandparents (**Grandparent 2** and **Grandparent 3**) of **parent 2** (referring to **Figure 3b**) were included at analyses to have a very general idea for distribution of phenotypical values. While analysing **Figure 3b**,It can be seen that traits namely; **ADL.NDF**, **HEM.NDF**, and **CEL.NDF** have average trait value (peak of the graphs represent average value for the given trait) between the range of the grandparents. This could show that segregation took place for overall population. More interestingly mapping population for traits **ADL.NDF** and **CEL.NDF** follow similar trend of distribution meanwhile trait **HEM.NDF** following the inverse fashion. This observation also overlaps with the previous research being conducted by Xu et al., (2012). For instance, when grand parent 2 has relatively higher lignin (compared to the mapping population), lower hemicellulose, and high cellulose; in contrast to the grand parent 3 which has lower lignin (among mapping population) , higher hemicellulose and lower lignin. It is quite clear that grandparents having a pretty contrasting characteristics for the traits lignin, hemicellulose, and cellulose which is a desired characteristic for formation of mapping population (Collard et al., 2005). However, assumption cannot be made about the segregation at parents 1 and 2 since we did not have a required phenotypical data to make a clear conclusion out of this observation. The compositional relationship also can be confirmed by the Xu et al., (2012) that cellulose and lignin had synergistic effect at saccharificaiton efficieny. Furthermore, increased hemicellulose levels could lead to decreased cellulose and lignin content as well.

When **ADF.DM**, **ADL.DM**, **CEL.DM**,**HEM.DM**, and **NDF.DM** were analysed together, it has been seen that variation among grandparents are still there but with narrowed range except **ADL.DM** (Figure 3b). Low scored prediction value (R^2= 0.3607) for lignin can explain the wider variation observed. Furthermore, it also shows that parents having quite contrasting lignin content. Mapping population formed from these parents also show quite variation some plants scoring higher or lower lignin content compared to the grandparents. Sole source of measured lignin is the cell wall; therefore, normally measured dm and ndf values for lignin should not vary that much. When comparison made between **Figure 3a-b** and **Figure 3b-k**, it has been seen that the validity of expectation above confirmed by showing the variation at very similar trend in lignin (ADL amounts. However, this is not the case for other cell wall components such as cellulose and hemicellulose. Plausible explanation would be that non-cell wall components such as, soluble sugars, proteins, lipids ,and starch could potentially contribute for the measured hemicellulose, cellulose amounts per gram of sample while lignin amount is more less stable since majority of lignin is coming from the cell wall and not from the non-cell wall components. Considering the fact that majority of released glucose comes from cellulose in the cell wall, one could expect to see similar glucose release (**CelRel**) trend for two grandparents But this expectation is valid under the assumption that harsh pre-treatments were used which could directly extract available glucose from the cell wall. In this experimentation, mild pre-treatment was used to point out real genotypic difference among the mapping population. However, When Figure **3b-g** and **3b-J** were compared, inverse trend has been observed in which Grandparent 2 having less cellulose (compared to Grandparent 3) but having high glucose release same as Grandparent 3 having more cellulose and having less glucose release relative to the grandparent 2. As a result, it could be said that glucose release is dictated by the level of biomass recalcitrance of the sample. Observed difference between the two groups (**NDF** vs **DM**) could be attributed to soluble sugars, protein, and starch which are not part of cell wall components

2.2 Phenotypical analysis of the mapping population continued with Year 2

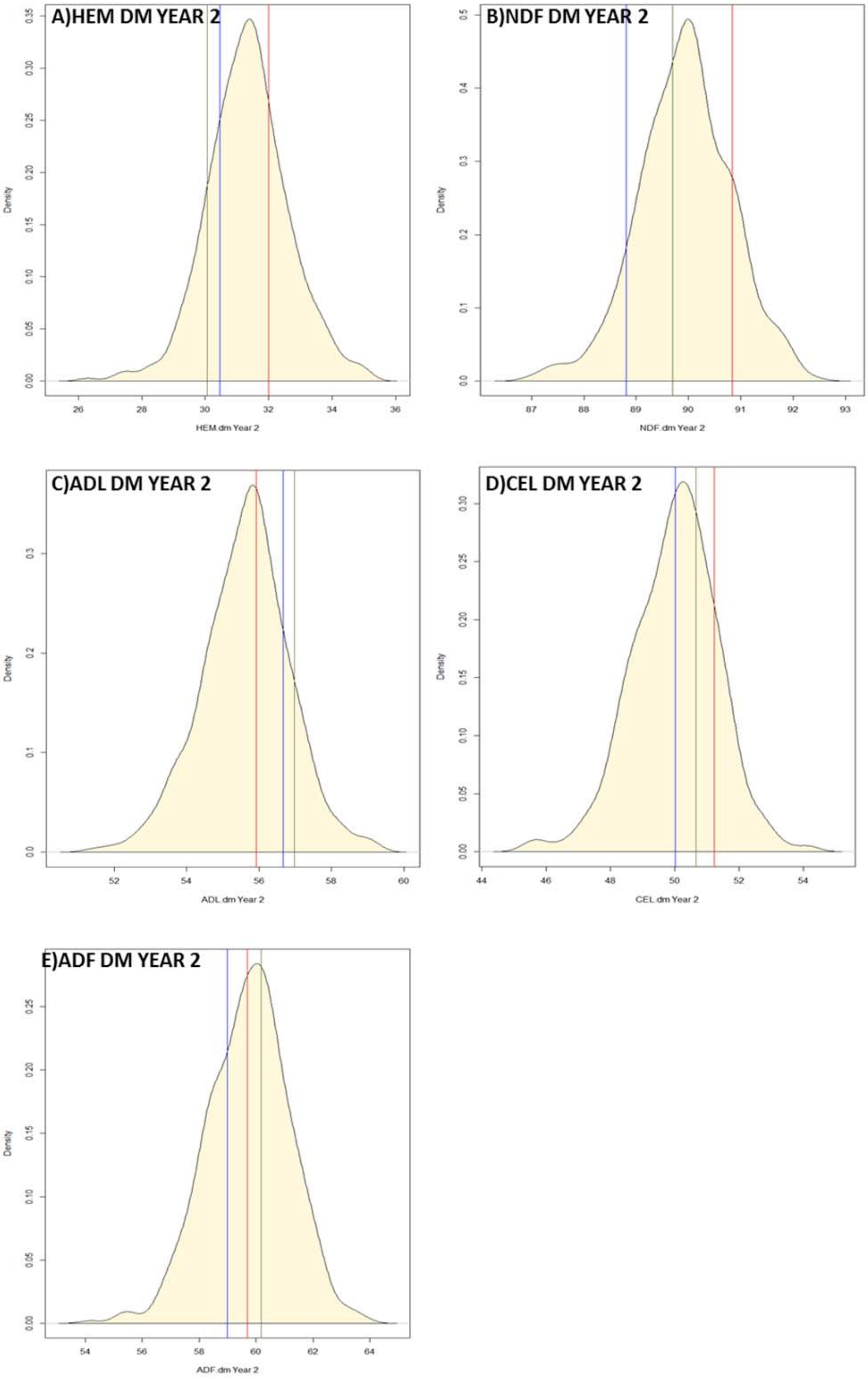


Figure 4a. Density curve for different cell wall traits including HEM, NDF,ADL,CEL and ADF for dry matter content (dm) in Year 2 where blue line being **Grandparent 2 of parent 2**, Red line representing the **parent 1**, and green being **Grandparent 3 of Parent 2.**

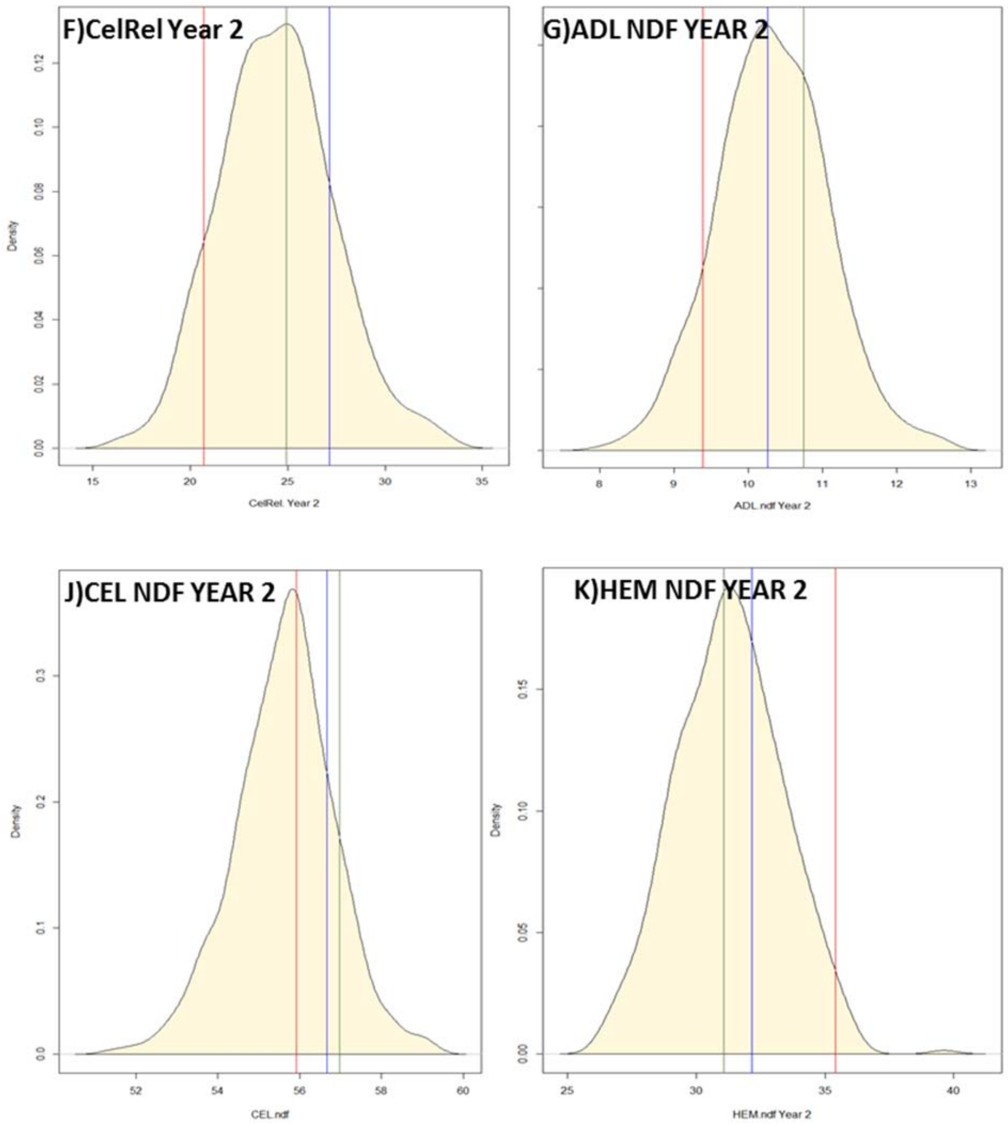


Figure 4b. Density curve versus different cell wall traits such as CelRel, ADL, CEL, and HEM for year 2, while blue line being **Grandparent 2 of parent 2**, Red line representing the **parent 1,** and Green being **Grandparent 3 of Parent 2**.

Regarding second year experimentation, phenotypical data for **parents 1** was obtained and for **parent 2** grandparents were used again. Assuming an average phenotypical value of grandparents taken as a representitative value for parent 2, then it could be said that mapping population performed between the range of parents which supports segregating population in second year. Interestingly for **Figure 3a-a, and Figure 3a-c Year 1,** it can be said that overall mapping population underperformed and over performed compared to grandparents for these traits which can be explained by transgressive segregation. Overall it could be said that for second year samples (referring **Figure 4a and Figure 4b**) some plants were performed better than the parents and some underperformed compared to the parents. As a result, transgressive segregation had occurred since the range of the population is larger than the difference between the two parents for the second year.

When comparison was made among **Figure 4b** -**G) ADL NDF YEAR 2, j)CEL.NDF YEAR 2,** and **K)HEM.NDF YEAR 2**, increased hemicellulose versus decreased lignin and decreased cellulose trend (as has been seen at the year 1) has also been observed for parent 1 (year 2). When closer look was made for trait **Figure 4b**- **F)CelRel Year 2,** average of the off springs are between the parents and considering the fact that released glucose is coming from cellulose we expect to see similar trend at **Figure 4b**- **J (CEL.NDF YEAR 2)** and which is also the case for the average value being between the values of parents for **Figure 4b-J (CEL.NDF YEAR 2).** When deeper analysis was made continuing with **Figure 4a-C) CEL DM YEAR 2 ,** it can be seen that complete inverse correlation was seen by parent 1 being the highest number instead of being the lowest at the NDF experiments. The large change at the trait for the parents (in NDF being the largest meanwhile in DM being the lowest or vice versa for grandparents) can be attributed to soluble sugar which could potentially compensate for the difference. In conclusion, it could be said that parents having extremely contrasting cell wall composition and saccharification efficiency. Furthermore, regarding both years, some plants are performing better and worse off than the parents related to the traits investigated which is an indication of transgressive segregation. Also, importance of non-cell wall components have been seen which could be having an impact on the traits investigated. Lastly, having high cellulose does not really mean high SE, showing that SE is dictated by recalcitrance.

3.Statistical Analysis was investigated for genotypes, year, and how each genotype affected from year affect.

Table 4. **Cell wall traits analyzed through one-way Anova with factors such as Genotype(G), Year(Y), and Year-genotype interaction (G*Y) for Dry Matter (DM) contents**

Trait	Factor	M.S.	F-prob
ADF%dm	Genotype (G)	9.425	<.001
	Year (Y)	7382.952	<.001
	G x Y	3.243	<.001
	Residual	1.399	
ADL%dm	Genotype (G)	2.9587	<.001
	Year (Y)	4510.8329	<.001
	G x Y	0.7514	<.001
	Residual	0.4764	
NDF%dm	Genotype (G)	5.3118	<.001
	Year (Y)	1177.2848	<.001
	G x Y	3.0847	<.001
	Residual	0.8631	
CEL%dm	Genotype (G)	7.456	<.001
	Year (Y)	823.059	<.001
	G x Y	2.488	<.001
	Residual	1.122	
HEM%dm	Genotype (G)	7.0566	<.001
	Year (Y)	1267.3668	<.001
	G x Y	1.2049	<.001
	Residual	0.6331	

Table 5. **Cell wall traits analyzed through two-way Anova with factors such as Genotype(G), Year(Y), and Year-genotype interaction (G*Y) for cell wall components (NDF).**

Trait	Factor	M.S.	F-prob
CelRel%	Genotype (G)	21.049	<.001
	Year (Y)	77120.63	<.001
	G x Y	8.027	<.001
	Residual	5.988	
HEM%ndf	Genotype (G)	11.757	<.001
	Year (Y)	11510.061	<.001
	G x Y	3.481	<.001
	Residual	2.269	
ADL%ndf	Genotype (G)	2.279	<.001
	Year (Y)	2807.8206	<.001
	G x Y	0.3704	<.001
	Residual	0.2425	
CEL%ndf	Genotype (G)	6.3165	<.001
	Year (Y)	193.6511	<.001
	G x Y	1.0822	<.001
	Residual	0.6168	

Genotypes (G) among the mapping population were found to be significantly different (<.001) among different traits shown above at table 4. Furthermore, comparing the year affect by analysing **Year 1** versus **Year 2**, significant difference has also been seen among the population by F value scoring (<.001) for Year factor (Y). Finally, **Genotype by Year interaction (G x Y)** also shows a significant interaction for all cell wall traits listed above (G x Y <.001). This can be translated as that some plant genotypes are affected differently from the year than the others. Mentioned significant differences have confirm that segregation had taken place at the mapping population for all cell wall traits.

First of all, with regards to Genotypes (G) affect, it was expected to see significant difference among the population since each individual plant should have a unique genotype due to segregation. Above results confirms the expectation made by showing significant difference among the genotypes. Secondly, year was also projected to have a significant impact on the mapping population due to change in plants physiology in two years (plants were grown more and reaching different maturity level in second year compared to the first year). Also, change in environmental conditions during year 1 and year 2 would have an impact on plant phenotype (considering the fact that plantation was made open field not in a greenhouse thereby changing environmental factors can have a direct impact on the traits). Year factor (Y) also shows a significant impact as has been expected. Thirdly, genotypes by year interaction shows that some genotypes have a different interaction with year than the others which also proves that there are unique genotypes in the mapping population. In another way, some plants were affected differently from the year than the others so year had an different impact on some genotypes than the others.

Table 5. **Summary statistics of the different cell wall components in %dm (dry matter) for two years including calculated heritability's**

Population statistics

Trait	**Year**	**Parent 1**	**G-Parent 2**	**G-parent 3**	**Mean**	**Min**	**Max**	**Range**	**LSD**	**Heritability**
ADF%dm	1		57.95	50.32	54.66	46.23	60.99	14.76	1.937	0.762(L*)
	2	59.07	59.00	60.17	59.73	54.18	63.83	9.65	1.834	
ADL%dm	1		7.14	4.62	6.36	4.00	9.65	5.651	0.915	0.842
	2	9.38	10.04	11.17	10.32	7.51	13.42	5.91	1.242	
CEL%dm	1		50.91	44.60	48.33	40.53	53.13	12.60	1.665	0.844
	2	51.23	50.02	50.65	50.00	45.36	54.18	8.82	1.691	
HEM%dm	1		32.58	33.39	33.42	30	37.12	7.112	1.088	0.899(H*)
	2	31.99	30.46	30.07	31.35	26.29	35.13	8.84	1.390	
NDF%dm	1		90.13	83.56	87.92	80.61	91.73	11.12	1.744	0.838
	2	90.84	88.81	89.70	89.94	86.97	92.42	5.449	1.188	

*(L indicating the lowest) *(H indicating the highest)

Table 6. **Summary statistics of the mapping population solely for cell wall components (NDF) in two years including calculated Heritability values**

Population statistics

Trait	**Year**	**Parent 1**	**G-Parent 1**	**G-parent 2**	**Mean**	**Min**	**Max**	**Range**	**LSD**	**Heritability**
ADL%ndf	1		7.52	6.45	7.19	5.08	10.04	4.959	0.755	0.888(H*)
	2	9.39	10.26	10.75	10.33	8.04	12.61	4.57	0.795	
CEL%ndf	1		56.26	53.07	54.85	50.07	58.13	8.06	1.129	0.804
	2	55.92	56.67	56.98	55.63	51.38	59.19	7.81	1.380	
HEM%ndf	1		35.12	41.47	37.64	32.12	44.14	12.02	1.992	0.799
	2	35.40	32.16	31.05	31.3	26.29	39.62	13.32	2.694	
CelRel%	1		38.93	49.55	40.95	31.71	54.88	23.17	3.379	0.747(L*)
	2	20.68	27.14	24.95	24.45	16.3	33.34	17.04	3.629	

Considering both **Table 6** and **5,** it can been said for the traits investigated, high heritability values have been observed, the lowest for ndf samples being the 0.747 while 0.762 being the lowest for dm samples. In general, heritability score being higher than 0.4 (40%) indicates that the selection for the trait in the population is usefull Therefore, with regards to the heritability, cell wall traits investigated can be usefull for further breeding programs. Furthermore, for both tables least significant differences (LSD) were calculated which showing a significant threshold level for comparison of means. Randomly or specifically selected genotypes from the mapping population can be evaluated based on LSD numbers whether they are significantly different from each other or not for the cell wall traits investigated.The more observations we have (more replicates/more blocks in our case), the less the variance should be and more precise would the observation be. Furthermore, the field environment where experimentation took place desired to have a more uniform physical characteristics which could lead more precise calculation of heritability. According to the **Formula 12,** heritability calculations were made for the cell wall traits investigated. It has been found that rougly 70-80% of the variation is due to differences in genotype and 20-30% is due to non-genetics factors (mostly due to environment). In most cases, it is desirable to reduce environmental effect on phenotype as much as possible therefore high heritability is one of the desired characteristics for investigated traits. Considering future breeding tasks, it is more desirable to minimize environmental impact on the trait of interest selected. Therefore, traits chosen and investigated seems to offer promising values with regards to heritibality for the traits. In conclusion, both table 5 and 6 gives the summary of the mapping population. Important conclusion could be made by looking at the table closely (indicated by circules), from year 1 to year 2 lignin amounts have increase; meanwhile, CelRel has drastically dropped meaning that lignin having a recalcitrance impact.

Formula 12

Below formulas have been used to calculate heritability values for the cell wall traits.

$$\sigma_G^2 = \frac{Mean\ Square\ Treatment(MST) - Residual(Error)}{Number\ of\ replicates(Block\ \#)}$$

$$\sigma_E^2 = \frac{Residual\ value(Error)}{Number\ of\ replicates(Block\ \#)}$$

$$H^2(Heritability) = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_E^2}$$

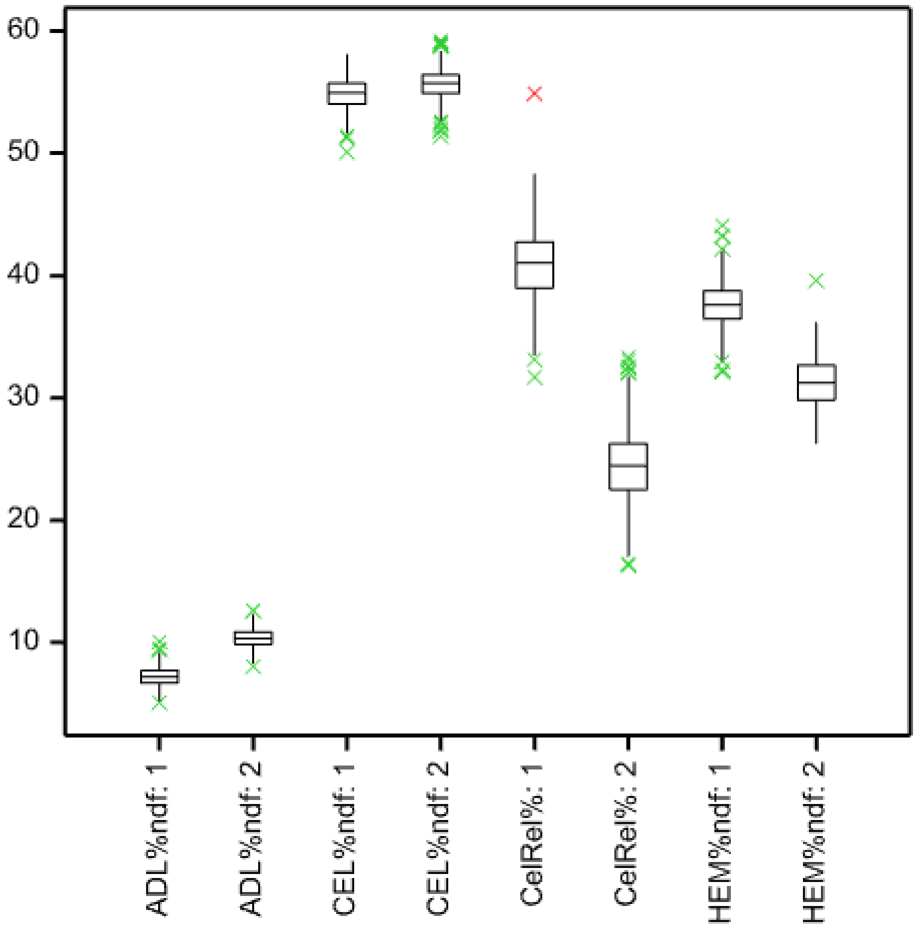


Figure 5. Comparison of cell wall traits in NDF associated with Year 1 and 2

When comparison was made between year 1 and 2, it can be seen that lignin, and hemicellulose levels were increased; however, inverse trend was observed for CelRel% and Hem%ndf. As has been mentioned earlier, based on different research (Xu et al.,2012,Allison et al.,2011), increased cellulose and lignin can lead to decrease hemicellulose. Two expected possibilities related to the amount of released glucose sugar are that first one being the amount of glucose sugar and the second being the extractability/digestibility of the available glucose. Based on the results obtained above, it is clear that the available glucose sugar did increase since cellulose amounts has risen in the second year. However, increasing cellulose levels did not lead to higher SE which could be due to low digestibility (leading to less accessibility of enzymes to cell wall sugars) resulted due to increased lignin levels in the second year which could prevent enzymes' access to cellulose thereby resulting with decreased CelRel%.

As has been explained earlier, CelRel is calculated by the amount of released glucose from the plant cell wall. Released glucose comes from the cellulose which was measured as part of saccharification efficiency experiment. Saccharification efficiency is a quite complex trait. One trait such as cellulose in cell wall could influence positively; however, there are lots of different factors which could also be negative factors (Xu et al.,2012). Furthermore, lignin's dominant negative impact on saccharification efficiency , secondly cell wall architecture and finally crystallization(Xu et al.,2012 (Arantes et al.,2010)(Lionetti et al.,2011) .The potential answers to the observation can be extended in which research leads more questions than the answers; meanwhile, considering the time limitations of the thesis several explanations will be addressed.

It has been known that there is a natural balance at the cell wall composition in which increase in one factor can result in decrease in another. This could explain 16.8 % decrease at the hemicellulose which could be the outcome of the increased lignin. So overall, decrease in cellulose may automatically increase percentage of hemicellulose but this is a valid assumption if one of the variables are being converted into another variable. In some cases, lignin amount may not be even really changing but due to decrease in hemicellulose it would seem as if it is increasing. This discourse could be more valid especially if these sugars are being degraded and converted into non-cell wall components such as soluble sugar under stressed conditions. However, in order to have a clear conclusion, plants grown in drought or being exposed to cold should be investigated. It is important to know which mechanism have a dominant cell wall determining role. In order to answer to this question physiological change taken place at different years should be investigated which is not in the scope of this research. According to Xu et al (2012), hemicellulose having a dominant impact shaping the balance on saccharification efficiency. Furthermore, considering the lesser amount of lignin extracted from the cell wall which is about 14% of all cell wall (Souza et al.,2014) but high increased at recalcitrance impact, it could concluded that lignin does not have a quantitative but a strong qualitative effect on saccharification efficiency

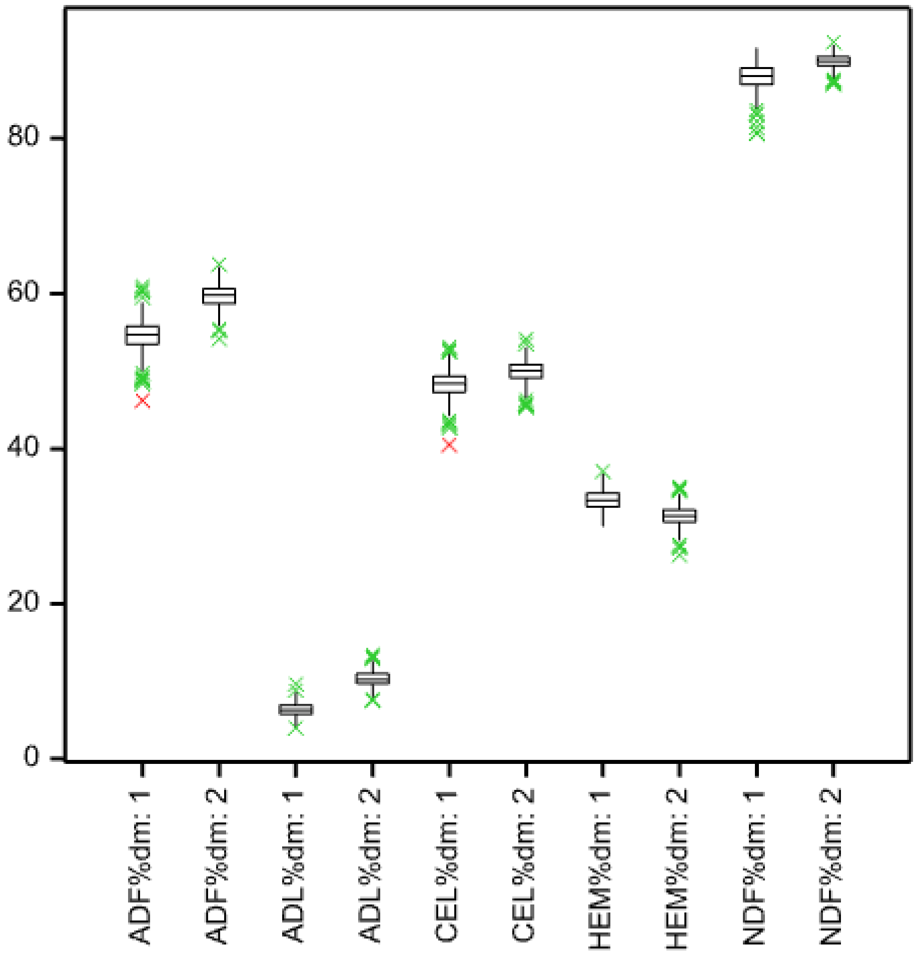


Figure 6. Change in cell wall dry matter traits in two years

ADF%dm,ADL%dm, Cel%dm, and NDF%dm contents were increased in Year 2 compared to Year 1. Hem%dm has decreased. Considering the plant growth and maturation, it is plausible to see increase at overall cell wall and non-cell wall components.

While analysing NDF% in two years (referring **Figure 6**), it has been seen that minor increase (2.02%) took place from 87.92% (**Year 1**) to 89.94% (**Year 2**). As has been known that terrestrial plants are sessile and had to endure with the change in the environment. They should be able to adapt themselves to changing environment to certain aspect and one of the ways of doing this comes from the use of non-cell components such as soluble sugars. Change in non-cell wall materials can be explained by that plant adapting to the environment that has been growing in by mobilizing its cell wall reservoirs.

According to Souza et al. (2015), *Miscanthus Sinesis* cell wall constituents consists of from cellulose, hemicellulose, and lignin combined value adding up to 86% of the cell wall while 14 percent being the non-cell wall components including ashes(minerals), soluble sugars etc. Minor increase in NDF in two years could mean minor drop in non-cell wall components(plant does not see a need to mobilize its non-cell wall/cell wall components), also could mean minor increase in overall cell wall (which could be an indication of minor growth). First of all, it could mean that plants could be using little bit of its non-cell wall reservoir adjusting itself to its surrounding since environment that they are growing is easy to adjust to, predictable and favourable for its growth. In addition to this, minor increase in NDF might also be translated as that biomass growth might have been activated. In order to explain the minor change in NDF, Mischanthus' true NDF capacity should be researched by different research data based on drought and cold tolerance of Mischanthus. According to Weijde,(personal communication)(2016), Domon et al.,(2013), Patton et al.,(2007) Van Kruijen (personal communication) Mischanthus do have high adaptability to cold and dry environments in which larger fluctuations at NDF values were observed. For instance, during a cold tolerance experiment, total NDF values dropped which could be translated also as increased as a difference between **DMC**-**NDF**=increased (in cold)) .Therefore having low increase in NDF can be directly attributed to the uniform environment of the mapping population grown also with the growing of the plant (increase maturity of plant). Furthermore, considering overall high heritability values calculated for Mischanthus, one can say that majority of change taken place in phenotype is due to genotype. In conclusion, both factors plants genotypic capacity (which could just also be given to the normal growth of the plant) and uniform environment directly influences minor change in NDF.

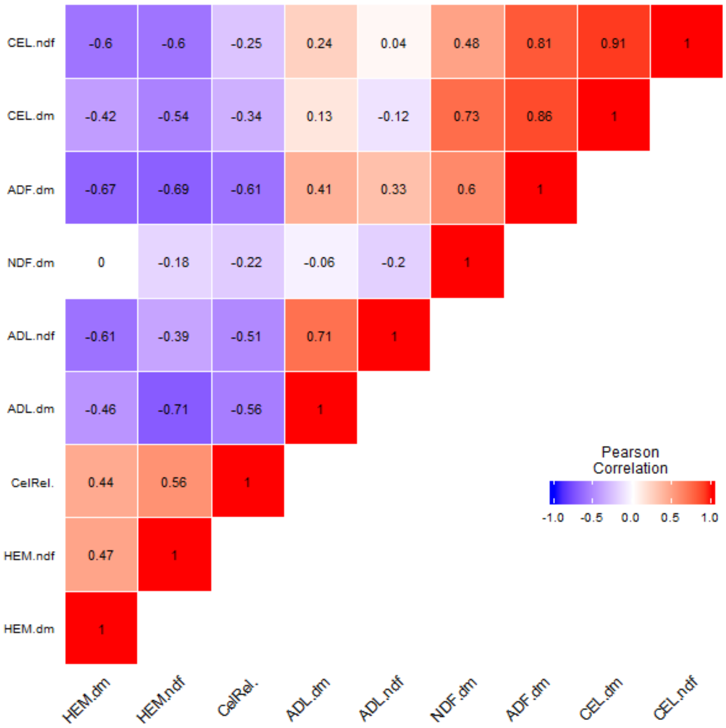


Figure 7. Correlation Analysis of cell wall components for the mapping population

According to the **Figure 7**, correlation among **lignin** (ADL.dm) and **cellulose** (Cel.dm) was found to be quite weak as **0.13** and it was even weaker when **lignin** (NDF) and **cellulose** (NDF) traits were compared scoring as **0.04**. According to Allison et al., when a different *M.sinensis* population analyzed in Wales, stronger correlation was observed between lignin (ADL.dm) and cellulose (Cel.dm) being **0.46** (2011). Further correlation comparison between **lignin** (ADL.dm) and **hemicellulose**(Hem.dm) has founded as **-0.46** in our experiment which is very similar to **-0.40** obtained by Allison et al.(2011). One of the major differences between the two research results was about the lignin. However, overall comparison among years can be conducted to give us a general trend change at the cell wall composition. Based on the experiment conducted in Wales, predicted mean for the **lignin** (ADL.dm) for three consecutive year (2006-2008) was not significantly different (P>0.001). In contrast, as has been explained earlier, our result shows that there has been a significant difference at the lignin levels in two years. Furthermore, calculated average **cellulose** was higher in our experiment (**48.33**—Year 1, **50.00**—Year 2) compared to the three yearlong cultivation 2006--**42.21** , 2007—**43.27**, 2008—**42.08** (Allison et al.,2011). In both experiments **hemicellulose** has significantly dropped during the years. Regarding NDF values, our field had a greater increase as +2.02% opposed to -1.34% drop of NDF in Wales. Different environmental conditions between Wales and Netherlands especially during the years of plantation could be accountable of some of the changes at the cell wall composition. Due to unfavorable environmental conditions especially in years 2007 (heavy rain fall) and 2008(strong wind, low temperature, snow) plants may not able to expand its cell wall instead could focus on its survival. Based on the climatic data of Wales from 2008, it has been seen record low temperature, heavy snow, and strong wind exposure (Website reference, 2016). As has been mentioned earlier, drop in NDF, cellulose, and hemicellulose could mean the degradation of stored polysaccharides furthermore non-cell wall components including soluble sugar could be mobilized to cope better with harsh climate conditions that plants endure for the population. In conclusion, changes between two population can be due to numerous factors. Knowing that the population investigated here in Netherlands having a high heritability could mean that environmental impacts having lesser effect compared to the genotypic impact. For better comparison, heritability values from Wales population should be known as well. However, it is quite clear that there are distinct phenotypical differences among the populations; meanwhile, major cell wall mechanisms such as decrease in hemicellulose (Referring figure 7) are intact in both cases

In conclusion, prediction model was developed for cell wall composition and saccharification efficiency for two years. Among the mapping population transgressive segregations were observed. Furthermore, large variation has been seen at the offspring's. Significant effect of year on phenotype was seen. It was also seen that genotypes were significantly different from each other. Each genotype had affected differently from year than the others. Large number of candidate QTLs were observed but not included in this report. Among some of the potential QTLs, co-localization was seen on the same QTL. The preliminary QTL report had been obtained and presented during the final presentation but due to time constraints the results has not been included in this report.

Future Research

Prediction model should be improved especially for complex traits such as CelRel. Furthermore, phenotyping for complex traits such as saccharification efficiency could be improved for better QTL mapping. Obtained preliminary QTL's could be validated. In future it could be investigated that how two or more QTLs interact with each other affecting the cell wall composition. Especially observed phenotypical correlation between cellulose & lignin versus hemicellulose can be traced at QTLs. Furthermore, identified QTL s degree of dominance can be investigated as well by seeing if the QTL alleles at the same locus interact (for instance, additive, dominant, recessive, or over-dominant). In addition, it could be investigated if these QTLs also do have an impact on other miscanthus varieties such as *Miscanthus Gigantaeus*, or *Miscanthus Sacchaleroflrous*

Identified QTL regions could be used for developing markers for marker assisted selection (MAS). As an alternative to QTL, genome wide association mapping (GWAS) can also be used by examining single nucleotide polymorphisms related to cell wall composition. Specific genome editing can be implemented at a target area with insertion, replacement, or removal of genes by using molecular scissors such as zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs), and the CRISPR/Cas system(Koh et al.,2015).

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5.Appendixes

Biochemical Lab data versus predicted lab data for cell wall composition and saccharification efficient

				Predicted	Lab	Predicted	Lab	Predicted	Lab	Predicted	Lab	Predicted	Lab
E_nr!	Block	G_name	Plot CK12(	NDF%dm	NDF%dm	ADF%dm	ADF%dm	CEL%dm	CEL%dm	HEM%dm	HEM%dm	ADL%dm	ADL%dm
6	1	SNL003	12	89.35	89.26	54.62	54.23	49.27	49.27	34.99	35.03	4.76	4.96
11	1	SNL008	6	87.39	86.94	53.52	53.52	48.45	48.85	33.29	33.42	5.33	4.67
12	1	SNL009	35	88.65	88.37	56.21	54.85	48.94	47.93	31.14	33.51	7.77	6.92
13	1	SNL010	66	86.98	87.14	53.58	53.59	47.52	47.09	34.25	33.55	6.08	6.50
14	1	SNL011	9	86.50	85.78	53.71	53.10	46.57	46.47	31.69	32.68	6.04	6.64
17	1	SNL014	4	88.56	88.44	54.46	56.51	48.15	50.07	33.91	31.93	6.53	6.45
19	1	SNL016	107	86.66	86.31	52.88	52.16	47.89	46.74	33.43	34.15	4.08	5.42
20	1	SNL017	196	86.59	86.26	54.20	54.05	47.86	48.31	32.52	32.21	5.64	5.74
24	1	SNL021	74	83.70	83.96	53.92	54.65	47.81	47.92	30.01	29.31	5.32	6.73
26	1	SNL023	18	84.20	85.00	52.03	51.88	46.52	47.14	31.94	33.13	5.81	4.74
31	1	SNL028	215	89.30	89.51	55.25	56.07	47.79	50.73	34.66	33.44	6.92	5.34
32	1	SNL029	43	90.29	89.59	57.60	57.25	51.57	50.72	32.82	32.33	6.35	6.53
33	1	SNL030	199	87.45	86.69	56.07	55.57	48.95	48.33	31.64	31.12	7.08	7.24
39	1	SNL036	182	86.41	87.10	54.13	54.59	48.11	48.76	32.20	32.51	5.07	5.83
44	1	SNL041	70	87.12	89.00	54.10	55.24	47.89	50.55	33.94	33.76	5.76	4.69
45	1	SNL042	129	87.21	87.22	54.64	55.54	49.75	50.08	31.49	31.68	4.62	5.46
53	1	SNL050	166	87.54	88.02	51.83	54.13	47.39	48.26	35.83	33.89	4.79	5.87
54	1	SNL051	147	86.54	86.40	54.46	53.96	47.95	47.85	30.63	32.44	6.11	4.07
55	1	SNL052	84	91.00	90.65	55.65	55.15	49.67	49.38	35.29	35.50	5.58	5.77
57	1	SNL054	175	88.32	87.47	56.09	56.63	48.91	50.14	32.21	30.84	6.94	6.49
64	1	SNL061	54	86.90	87.12	54.66	54.12	48.50	49.01	31.93	33.00	6.07	5.11
66	1	SNL063	191	88.02	88.85	54.33	53.85	47.85	47.98	33.47	35.01	6.48	5.87
69	1	SNL066	164	87.17	87.28	54.67	55.87	49.57	50.15	32.08	31.40	6.22	5.72
71	1	SNL068	16	87.40	86.87	54.49	56.38	48.74	49.22	32.41	30.49	5.67	7.16
75	1	SNL072	81	91.08	90.88	55.91	55.46	49.80	49.47	35.33	35.42	6.04	5.98
76	1	SNL073	62	90.37	90.28	55.47	55.94	49.47	50.35	35.06	34.34	5.31	5.59
77	1	SNL074	209	87.09	87.22	54.54	54.04	48.71	47.35	32.50	33.18	5.91	6.69
82	1	SNL079	192	86.16	86.45	52.82	53.06	48.26	47.74	32.98	33.39	4.29	5.32
85	1	SNL082	60	88.28	87.83	55.67	56.94	49.72	50.34	31.53	30.89	6.15	6.60
88	1	SNL085	26	89.68	89.61	56.26	56.65	50.43	50.57	32.99	32.96	6.35	6.08
92	1	SNL089	201	86.95	87.15	54.79	53.86	48.84	48.18	32.36	33.29	6.10	5.68
93	1	SNL090	180	88.67	89.28	55.55	55.30	50.34	50.04	32.63	33.98	5.42	5.26
94	1	SNL091	142	90.67	90.11	57.45	56.39	52.52	49.01	31.40	33.72	6.48	7.39
98	1	SNL095	168	87.62	88.18	53.28	54.56	46.97	47.58	32.97	33.62	5.69	6.98
107	1	SNL104	133	88.73	88.08	56.92	57.30	50.41	49.98	31.58	30.78	6.62	7.32
110	1	SNL107	127	88.63	88.18	55.75	55.01	50.93	48.65	32.75	33.17	6.38	6.36
114	1	SNL111	69	88.76	89.17	55.62	54.77	49.99	49.24	33.39	34.40	6.39	5.53
115	1	SNL112	183	89.13	88.79	57.18	58.20	48.88	50.29	32.21	30.58	7.86	7.91
121	1	SNL118	28	89.50	87.68	56.66	56.16	51.24	50.53	33.52	31.52	6.03	5.64
122	1	SNL119	96	88.07	87.76	54.27	54.64	48.69	49.23	33.33	33.11	5.85	5.41
125	1	SNL122	55	86.27	87.24	54.26	54.99	48.31	49.66	32.16	32.26	5.87	5.33
127	1	SNL124	205	88.72	88.83	55.74	56.54	49.94	50.59	33.26	32.29	4.86	5.95
130	1	SNL127	216	86.68	87.46	54.10	54.71	47.44	48.81	33.10	32.75	5.78	5.90
132	1	SNL129	25	88.94	88.94	52.51	53.09	47.14	47.34	36.47	35.85	4.87	5.75
133	1	SNL130	79	87.65	87.02	53.42	53.38	48.73	48.28	33.29	33.64	4.23	5.10
140	1	SNL137	93	88.70	88.53	56.45	56.79	49.54	49.16	32.76	31.74	7.00	7.63
142	1	SNL139	125	91.37	90.75	59.56	58.63	53.13	52.22	31.16	32.13	6.60	6.40
143	1	SNL140	56	89.10	88.32	57.66	57.28	51.66	51.77	31.35	31.04	5.54	5.50

145	1	SNL142	126	89.11	90.52	54.03	53.64	48.41	48.36	35.43	36.88	5.76	5.28
149	1	SNL146	177	88.60	88.94	55.94	56.13	50.75	50.60	31.33	32.81	6.09	5.52
150	1	SNL147	42	88.40	87.63	55.11	54.86	48.70	48.90	32.40	32.78	6.64	5.96
151	1	SNL148	172	86.55	87.18	53.39	53.99	48.01	48.98	31.97	33.18	5.09	5.01
154	1	SNL151	65	86.60	87.02	53.50	53.64	48.81	48.49	32.30	33.38	5.26	5.15
155	1	SNL152	85	85.95	85.93	52.71	52.16	46.28	46.11	33.70	33.77	5.55	6.05
161	1	SNL158	145	88.08	87.32	55.06	54.02	49.78	48.71	33.95	33.30	4.23	5.31
165	1	SNL162	212	84.46	84.81	50.93	51.40	44.52	46.34	33.94	33.41	6.15	5.06
166	1	SNL163	14	89.76	88.92	58.11	57.99	51.98	52.29	31.48	30.93	6.12	5.71
174	1	SNL171	10	83.86	84.05	51.32	50.94	45.19	45.61	32.21	33.11	5.02	5.33
175	1	SNL172	103	86.52	86.59	54.62	54.65	48.56	49.06	32.18	31.94	5.67	5.59
176	1	SNL173	203	87.27	88.76	51.48	52.03	46.39	46.50	35.90	36.73	5.24	5.52
177	1	SNL174	214	82.17	80.75	50.10	48.88	44.72	44.36	33.21	31.87	4.54	4.52
179	1	SNL176	104	89.08	89.54	55.75	55.46	50.89	50.14	33.60	34.08	5.76	5.33
181	1	SNL178	3	89.15	89.80	56.16	55.97	49.07	49.50	32.47	33.83	7.41	6.46
187	1	SNL184	178	88.50	88.75	55.90	55.22	50.76	50.19	32.52	33.53	5.21	5.03
193	1	SNL190	5	88.12	87.85	57.02	55.58	48.86	49.74	30.89	32.28	7.39	5.83
199	1	SNL196	122	88.69	87.88	57.55	57.29	51.63	50.49	31.13	30.59	6.26	6.80
7	2	SNL004	350	85.36	85.41	51.87	50.61	44.65	44.22	33.70	34.79	5.53	6.39
18	2	SNL015	398	87.50	88.17	52.38	51.55	45.47	45.13	35.67	36.62	6.49	6.41
20	2	SNL017	373	86.89	87.14	54.45	54.71	48.39	48.82	32.63	32.42	6.52	5.90
22	2	SNL019	428	87.85	89.32	54.79	55.42	48.51	49.15	33.95	33.90	6.46	6.27
26	2	SNL023	404	86.92	87.15	54.67	54.43	48.17	47.64	31.57	32.72	6.44	6.78
27	2	SNL024	334	87.73	87.86	56.00	55.39	49.30	50.20	32.05	32.47	7.68	5.19
29	2	SNL026	277	91.40	91.31	57.81	57.21	50.66	50.49	33.89	34.10	7.12	6.72
34	2	SNL031	421	88.29	89.36	53.59	53.14	46.83	46.89	36.48	36.22	6.08	6.25
39	2	SNL036	343	85.62	85.47	53.32	52.86	47.49	47.96	33.88	32.61	5.68	4.90
40	2	SNL037	274	89.54	89.69	56.38	55.43	48.69	49.57	32.57	34.27	7.97	5.85
42	2	SNL039	303	89.53	91.00	54.99	54.82	48.19	48.38	34.92	36.18	6.57	6.44
45	2	SNL042	242	83.76	85.37	52.30	52.20	46.69	46.81	32.08	33.17	5.69	5.40
48	2	SNL045	243	88.30	87.96	54.83	53.91	47.70	48.08	33.41	34.05	6.44	5.84
53	2	SNL050	232	87.11	87.50	50.84	52.39	44.74	46.00	36.82	35.11	5.87	6.39
68	2	SNL065	397	90.61	91.01	57.48	55.85	50.15	49.22	33.82	35.15	7.38	6.64
71	2	SNL068	314	88.63	88.79	55.77	56.44	48.71	49.54	33.06	32.68	6.05	6.90
73	2	SNL070	372	88.41	87.17	54.77	53.45	48.20	47.41	32.74	33.71	6.52	6.04
75	2	SNL072	425	89.24	89.94	56.30	56.57	48.23	48.60	33.47	33.37	7.53	7.98
89	2	SNL086	406	89.71	89.20	54.05	53.86	47.87	46.97	35.76	35.33	6.27	6.89
91	2	SNL088	286	88.33	88.77	53.34	53.25	46.68	47.30	35.95	35.52	5.54	5.95
98	2	SNL095	416	89.62	88.97	56.23	55.95	48.35	47.00	32.93	33.02	8.16	8.95
106	2	SNL103	233	90.78	91.37	56.57	55.58	49.28	49.59	34.55	35.79	6.96	5.99
115	2	SNL112	306	89.81	89.62	57.31	57.26	49.29	49.52	33.23	32.35	8.64	7.74
123	2	SNL120	252	87.06	88.65	54.06	55.04	47.01	49.00	33.75	33.61	7.14	6.05
125	2	SNL122	409	87.84	87.98	56.92	56.43	49.28	49.84	31.40	31.55	7.16	6.60
128	2	SNL125	262	87.65	87.91	54.44	54.17	47.78	49.37	33.04	33.74	7.43	4.80
136	2	SNL133	267	85.91	85.18	54.14	52.40	48.11	46.86	32.17	32.78	5.49	5.54
139	2	SNL136	322	87.39	87.90	53.59	54.59	47.95	47.85	33.67	33.32	6.12	6.74
140	2	SNL137	413	88.49	89.17	56.98	58.93	49.48	51.71	32.46	30.24	8.23	7.22
142	2	SNL139	400	91.61	91.06	60.99	60.19	52.32	52.37	30.45	30.87	8.36	7.82
149	2	SNL146	217	87.54	87.60	53.80	55.35	49.01	49.69	33.02	32.25	4.67	5.66
161	2	SNL158	315	89.21	89.11	55.23	56.05	48.85	49.12	34.40	33.06	5.59	6.93

173	2	SNL170	281	88.02	88.47	56.43	57.17	47.73	50.65	33.04	31.30	8.19	6.52
181	2	SNL178	241	90.15	89.71	57.73	57.02	50.93	50.07	32.15	32.69	6.96	6.95
183	2	SNL180	299	86.05	85.56	52.68	51.79	46.76	45.88	34.69	33.76	5.54	5.91
191	2	SNL188	283	84.09	84.66	51.45	52.46	46.04	47.57	33.43	32.20	5.39	4.89
199	2	SNL196	317	86.93	87.12	55.62	54.96	49.29	48.55	31.78	32.15	6.93	6.41
2	3	H0012	437	89.21	89.13	55.31	54.01	48.81	47.98	34.06	35.12	6.18	6.02
3	3	H0163	557	86.28	86.23	52.85	48.10	46.43	43.67	34.62	38.14	4.51	4.42
6	3	SNL003	447	86.90	89.43	51.40	51.75	45.79	47.29	36.80	37.67	4.62	4.46
7	3	SNL004	453	84.57	84.67	50.74	51.62	43.67	45.06	34.38	33.05	5.63	6.56
18	3	SNL015	558	90.00	90.76	57.09	56.62	48.85	48.90	33.68	34.14	8.34	7.72
19	3	SNL016	648	87.88	88.08	55.50	54.97	48.93	48.90	32.79	33.12	5.84	6.07
23	3	SNL020	621	80.72	80.00	48.89	47.81	43.35	43.35	33.87	32.19	4.89	4.47
35	3	SNL032	646	85.55	86.21	53.63	53.25	47.01	46.44	32.35	32.95	7.01	6.82
39	3	SNL036	555	84.32	84.22	53.22	55.40	46.51	49.04	33.06	28.82	6.69	6.36
45	3	SNL042	545	85.00	85.83	54.30	53.19	47.43	47.37	32.38	32.64	6.19	5.82
53	3	SNL050	592	87.04	85.89	51.51	49.81	46.51	44.65	36.02	36.09	4.90	5.15
54	3	SNL051	513	86.36	86.02	53.37	53.59	46.36	46.77	32.17	32.43	7.60	6.82
64	3	SNL061	445	87.83	87.54	54.50	55.15	48.40	48.62	33.81	32.39	6.33	6.52
72	3	SNL069	480	90.53	90.66	55.26	54.30	48.49	48.86	36.15	36.36	6.79	5.43
87	3	SNL084	524	88.19	88.03	53.85	54.84	47.70	48.25	34.33	33.19	6.30	6.59
98	3	SNL095	591	88.80	88.31	53.70	55.55	46.23	47.85	34.85	32.77	7.11	7.70
102	3	SNL099	612	86.02	86.49	51.99	53.20	46.00	47.35	35.03	33.29	5.72	5.86
112	3	SNL109	514	87.52	87.25	53.06	53.39	48.02	48.13	34.70	33.86	5.72	5.25
118	3	SNL115	585	86.95	86.81	55.39	55.36	48.94	48.27	32.09	31.45	6.23	7.08
119	3	SNL116	449	88.70	88.62	56.38	56.32	49.95	50.25	32.35	32.30	7.27	6.07
133	3	SNL130	604	86.25	86.23	52.51	52.77	47.54	47.30	33.89	33.46	5.06	5.47
134	3	SNL131	542	85.44	85.15	52.80	51.87	45.71	44.90	34.83	33.28	5.58	6.97
144	3	SNL141	635	83.81	84.27	48.75	50.43	42.76	44.79	35.79	33.84	5.62	5.64
146	3	SNL143	510	85.31	86.06	52.47	52.46	46.69	46.17	33.34	33.60	6.40	6.30
160	3	SNL157	601	87.57	87.99	53.99	53.59	47.60	47.28	33.79	34.40	6.20	6.30
168	3	SNL165	640	90.96	90.60	58.93	58.92	50.71	51.07	32.57	31.68	8.16	7.85
180	3	SNL177	492	88.03	88.31	53.35	54.38	47.41	47.61	34.17	33.93	5.89	6.77
183	3	SNL180	606	88.14	88.45	53.68	53.79	47.89	48.00	34.70	34.65	6.19	5.79
188	3	SNL185	576	89.11	89.70	56.42	55.67	48.51	48.25	33.38	34.03	8.25	7.42
193	3	SNL190	618	89.12	89.11	57.98	58.24	49.46	50.60	31.92	30.88	8.36	7.63
197	3	SNL194	614	86.06	85.87	51.04	51.03	44.72	44.09	35.72	34.84	5.99	6.93
200	3	SNL197	588	87.59	88.61	52.99	53.08	46.80	46.91	34.76	35.53	6.50	6.17

Predicted CEL%ndf	Lab CEL%ndf	Predicted HEM%ndf	Lab HEM%ndf	Predicted ADL%ndf	Lab ADL%ndf	Predicted CelRel%	Lab CelRel%
54.36	55.20	39.38	39.24	6.14	5.56	40.27	48.06
55.09	56.19	38.26	38.44	5.82	5.38	43.43	42.57
55.24	54.24	35.30	37.93	8.31	7.84	35.62	36.21
54.12	54.04	38.01	38.50	7.07	7.46	41.96	43.46
53.87	54.17	38.87	38.09	7.77	7.74	40.55	36.92
54.20	56.61	38.15	36.10	7.56	7.29	37.42	38.51
54.85	54.15	39.08	39.57	5.60	6.28	43.52	50.72
55.38	56.00	36.95	37.34	6.84	6.65	40.40	39.08
56.70	57.07	35.38	34.91	7.13	8.02	43.13	40.49
54.95	55.45	37.99	38.97	6.69	5.58	41.30	44.18
53.64	56.68	37.60	37.36	8.08	5.96	38.78	35.94
56.40	56.62	34.99	36.09	7.20	7.29	36.83	40.28
55.82	55.75	36.02	35.90	7.98	8.36	35.85	43.19
55.05	55.98	37.94	37.33	6.74	6.70	41.67	37.59
54.72	56.79	38.36	37.94	7.02	5.27	38.67	42.35
56.81	57.42	38.10	36.32	6.11	6.27	44.92	41.27
53.38	54.83	40.28	38.50	5.70	6.67	42.18	38.60
55.59	55.39	36.96	37.55	7.57	7.07	37.38	38.37
53.87	54.48	39.73	39.16	6.61	6.36	39.42	39.04
55.63	57.33	36.41	35.25	7.87	7.42	37.51	38.60
55.57	56.25	37.40	37.88	7.22	5.87	41.32	44.25
54.06	54.00	38.10	39.40	7.81	6.61	37.11	40.99
56.86	57.46	36.39	35.98	6.47	6.56	39.07	39.35
55.53	56.66	37.29	35.10	6.48	8.24	40.07	41.22
53.77	54.44	38.06	38.98	6.75	6.58	38.92	37.15
54.07	55.77	37.81	38.03	6.70	6.20	38.96	40.68
55.58	54.29	37.13	38.04	6.71	7.67	40.71	40.29
55.78	55.22	40.79	38.62	5.63	6.15	44.52	48.15
56.02	57.32	36.13	35.17	6.61	7.52	42.60	43.12
55.34	56.43	37.03	36.78	7.30	6.79	40.08	37.46
55.79	55.28	36.90	38.20	6.94	6.51	38.78	42.55
56.32	56.05	38.15	38.06	6.80	5.89	41.05	41.92
57.56	54.38	35.11	37.42	6.22	8.20	37.92	43.97
53.34	53.96	39.36	38.12	7.55	7.91	39.66	33.57
56.61	56.74	35.68	34.95	7.68	8.31	39.00	42.33
57.26	55.17	36.84	37.62	6.40	7.21	40.98	42.25
55.47	55.22	36.77	38.58	6.51	6.20	41.74	45.32
54.74	56.65	35.66	34.44	9.53	8.91	33.95	38.85
57.09	57.63	35.88	35.94	6.29	6.43	37.03	39.51
54.76	56.10	38.91	37.73	6.81	6.17	40.80	40.26
55.73	56.92	38.58	36.97	7.03	6.11	40.84	49.89
55.70	56.96	39.09	36.35	6.34	6.70	42.19	45.74
54.57	55.80	37.41	37.45	6.76	6.75	43.65	46.14
52.33	53.23	40.98	40.30	6.06	6.47	41.63	38.47
55.21	55.48	39.58	38.65	5.74	5.86	44.65	45.13
55.41	55.53	34.93	35.85	8.10	8.62	39.35	40.82
57.81	57.54	33.16	35.40	6.90	7.06	36.96	37.32
57.49	58.62	35.66	35.15	6.76	6.23	39.03	40.05

53.17	53.43	39.77	40.74	7.06	5.83	41.39	41.34
56.91	56.89	37.12	36.89	6.32	6.21	42.29	45.77
54.75	55.80	37.42	37.40	7.41	6.80	39.83	43.14
55.05	56.19	39.46	38.06	6.51	5.75	41.56	42.65
56.06	55.72	37.56	38.36	5.68	5.92	45.25	38.40
53.66	53.66	38.08	39.30	7.06	7.04	40.77	42.47
56.01	55.78	37.41	38.13	5.61	6.08	44.44	44.61
53.11	54.64	40.46	39.40	7.28	5.96	45.87	39.43
57.67	58.80	36.02	34.78	7.27	6.42	37.67	36.64
53.98	54.26	38.98	39.39	6.94	6.35	41.39	39.10
55.71	56.66	36.90	36.89	6.78	6.46	41.50	41.85
52.51	52.39	41.73	41.38	6.35	6.22	43.80	45.74
54.18	54.94	40.27	39.46	6.18	5.60	48.14	45.51
56.44	55.99	36.65	38.06	6.57	5.95	39.94	45.38
54.77	55.13	37.44	37.67	8.81	7.20	37.53	41.37
56.86	56.56	36.91	37.78	6.38	5.67	42.36	43.18
55.28	56.62	36.10	36.74	9.35	6.64	38.96	44.53
57.55	57.45	35.25	34.81	7.17	7.74	38.76	37.59
52.56	51.78	40.58	40.74	7.50	7.49	41.46	40.09
52.11	51.19	39.40	41.54	7.50	7.27	42.62	41.65
56.03	56.02	36.13	37.21	7.22	6.77	40.84	36.73
55.01	55.03	36.93	37.95	6.84	7.02	41.68	43.93
55.72	54.67	37.47	37.55	7.01	7.78	41.82	41.39
56.23	57.13	35.06	36.96	7.85	5.91	38.40	40.12
55.31	55.30	36.02	37.34	7.85	7.36	38.59	41.81
52.63	52.48	38.84	40.53	6.77	6.99	42.12	43.03
55.39	56.11	38.66	38.15	6.86	5.74	43.86	38.78
54.17	55.27	36.89	38.20	8.40	6.52	39.31	42.71
53.37	53.16	38.74	39.76	7.82	7.08	40.33	38.88
55.66	54.83	39.21	38.85	6.83	6.32	46.79	49.41
54.65	54.66	38.53	38.71	7.43	6.64	41.29	39.28
51.22	52.58	41.31	40.12	6.56	7.30	45.29	42.76
55.15	54.08	35.79	38.63	8.13	7.29	36.16	37.50
55.05	55.79	37.12	36.80	7.38	7.77	41.16	40.32
54.38	54.39	37.78	38.68	6.85	6.93	43.81	40.84
53.75	54.03	36.56	37.10	8.25	8.87	37.51	33.61
53.24	52.66	39.70	39.61	7.43	7.72	40.26	36.44
52.78	53.28	39.68	40.01	7.01	6.70	42.93	38.37
54.28	52.82	36.26	37.11	8.89	10.06	35.91	34.52
54.02	54.27	37.79	39.17	8.11	6.55	39.10	40.39
55.22	55.26	34.20	36.10	8.51	8.64	37.92	38.98
53.63	55.27	36.65	37.91	7.56	6.82	39.67	42.69
56.41	56.65	35.96	35.86	7.65	7.50	41.45	44.69
54.71	56.16	37.27	38.38	7.69	5.45	41.46	42.33
55.44	55.01	38.76	38.49	6.61	6.50	44.90	52.94
54.89	54.43	38.11	37.90	6.49	7.67	44.07	36.83
55.77	57.99	34.33	33.91	8.49	8.10	35.60	34.14
57.57	57.51	32.12	33.91	9.02	8.59	31.72	36.49
55.76	56.72	39.11	36.82	5.08	6.46	47.77	46.14
54.67	55.12	37.39	37.10	6.65	7.78	42.19	40.10
54.44	57.25	34.94	35.38	8.77	7.37	38.64	38.95
56.41	55.81	35.57	36.44	7.84	7.75	40.51	42.89
53.78	53.63	39.28	39.46	6.42	6.91	43.85	49.46
54.62	56.19	39.43	38.03	6.48	5.78	44.21	42.20
56.38	55.73	36.20	36.91	6.82	7.36	43.57	43.37
54.71	53.84	37.91	39.40	6.95	6.76	42.46	46.65
53.60	50.65	41.93	44.22	6.39	5.13	46.98	51.71
52.18	52.88	41.60	42.13	5.88	4.99	48.03	43.77
52.33	53.21	39.81	39.04	7.24	7.75	42.32	39.34
54.55	53.88	35.95	37.62	9.19	8.50	33.53	38.79
55.19	55.51	37.37	37.60	6.82	6.89	42.64	36.85
53.30	54.18	40.50	40.23	6.14	5.58	48.34	44.57
54.86	53.87	37.04	38.23	7.96	7.91	42.38	39.64
54.90	58.22	37.07	34.22	7.49	7.56	41.39	43.86
55.40	55.19	38.10	38.03	7.42	6.78	43.85	43.75
53.07	51.98	42.20	42.02	6.21	6.00	45.52	44.22
53.75	54.37	37.71	37.70	8.08	7.93	42.87	43.80
55.02	55.55	37.30	37.00	6.73	7.45	43.91	41.11
53.56	53.90	38.93	40.11	7.26	5.99	40.19	43.31
54.55	54.81	38.45	37.71	6.94	7.49	43.01	43.77
52.36	54.18	38.60	37.10	7.94	8.72	39.52	31.36
53.06	54.74	40.15	38.49	6.98	6.77	43.07	41.46
54.93	55.17	39.77	38.81	6.60	6.02	43.67	45.38
56.01	55.61	36.14	36.23	7.19	8.16	37.94	31.60
56.50	56.70	35.48	36.44	7.47	6.85	41.00	44.17
55.06	54.85	39.57	38.81	6.38	6.34	44.54	40.42
53.38	52.73	39.97	39.08	7.13	8.18	39.98	42.48
51.21	53.15	42.07	40.16	7.15	6.69	45.10	48.86
54.54	53.64	37.93	39.04	7.21	7.32	44.02	40.81
54.11	53.74	38.30	39.09	7.02	7.17	41.79	38.92
55.66	56.37	33.60	34.96	8.46	8.67	36.28	35.62
54.17	53.91	39.23	38.42	6.66	7.67	44.46	41.26
53.69	54.28	39.66	39.18	6.94	6.55	42.46	43.02
54.36	53.79	35.81	37.94	8.48	8.27	36.43	35.65
55.94	56.79	33.58	34.65	9.08	8.57	35.69	39.87
51.80	51.35	42.11	40.57	7.38	8.08	43.47	42.07
53.37	52.94	37.87	40.10	6.67	6.96	42.70	41.93

Phenotypical data obtained for two years for whole mapping population for different cell wall traits and saccharification efficiency related trait (CelRel%)

		NDF%dm	ADF%dm	CEL%dm	HEM%dm	ADL%dm	CEL%ndf	HEM%ndf	ADL%ndf	CelRel%
Year	E_nr!	Average	Average	Average	Average	Average	Average	Average	Average	Average
1	1	*	*	*	*	*	*	*	*	*
2	1	90.84	59.70	51.23	31.99	9.38	55.92	35.40	9.39	20.68
1	2	90.13	57.95	50.91	32.58	7.14	56.26	35.12	7.52	38.93
2	2	88.81	59.00	50.02	30.46	10.04	56.67	32.16	10.26	27.14
1	3	83.56	50.32	44.60	33.39	4.62	53.07	41.47	6.45	49.55
2	3	89.70	60.17	50.65	30.07	11.17	56.98	31.05	10.75	24.95
1	4	88.51	55.90	49.84	32.08	6.67	56.14	35.44	7.09	41.55
2	4	90.51	59.84	51.03	31.71	9.27	56.43	33.02	9.53	27.89
1	6	88.13	53.01	47.53	35.89	4.69	53.27	40.49	6.01	44.15
2	6	89.00	55.58	47.15	34.15	8.44	52.92	37.80	8.83	32.01
1	7	85.22	51.34	44.58	33.88	5.45	52.51	40.16	7.25	41.49
2	7	88.96	57.60	47.69	31.84	9.99	53.93	31.88	10.34	25.74
1	8	87.37	52.28	45.84	35.56	5.09	52.62	41.83	6.56	44.46
2	8	89.24	58.51	48.87	30.58	8.68	54.13	34.88	9.55	23.62
1	9	90.44	58.08	50.73	32.44	7.39	56.16	34.69	7.87	35.86
2	9	90.26	60.78	50.92	30.61	10.63	56.07	30.20	10.57	20.38
1	10	88.96	55.31	48.62	33.27	6.87	54.48	38.49	7.89	38.15
2	10	90.60	61.48	50.98	29.35	10.83	56.55	30.33	11.20	22.04
1	11	86.71	52.67	47.34	33.77	5.69	54.54	39.34	6.29	43.37
2	11	90.69	59.92	51.13	31.95	9.59	56.01	31.82	9.06	23.88
1	12	88.61	55.90	48.23	31.28	8.08	54.57	36.38	8.61	37.09
2	12	89.10	59.90	48.78	30.52	11.13	54.42	30.51	11.60	20.47
1	13	87.26	54.01	47.23	34.21	6.63	53.98	37.39	7.47	41.92
2	13	89.52	58.47	49.20	32.66	10.56	54.95	31.00	10.10	26.66
1	14	86.60	54.21	46.91	31.68	6.68	54.19	38.52	8.18	39.70
2	14	89.88	61.23	50.86	28.69	11.65	56.52	29.46	11.41	21.98
1	15	87.61	54.47	47.86	33.39	6.57	54.57	36.72	7.31	40.51
2	15	90.04	59.55	49.91	31.83	10.89	55.34	29.44	10.22	22.49
1	16	88.20	55.76	49.26	32.31	6.15	55.98	37.19	7.13	40.36
2	16	90.80	61.30	51.54	30.56	9.81	56.82	31.12	10.01	22.55
1	17	87.33	54.40	47.73	33.22	6.66	54.61	36.99	7.77	39.00
2	17	90.59	60.77	50.78	30.71	10.48	56.23	30.14	10.96	21.36
1	18	89.24	55.14	47.72	34.39	7.28	53.65	37.87	8.44	38.29
2	18	89.65	59.33	49.51	31.29	12.17	55.61	31.30	10.85	23.27
1	19	86.79	53.99	48.06	33.07	5.24	55.14	37.99	6.48	42.35
2	19	90.17	60.43	50.52	30.74	9.36	55.87	31.57	10.02	24.33
1	20	87.16	54.69	48.39	32.67	6.54	55.72	36.12	7.17	40.66
2	20	89.78	60.43	50.33	31.12	11.40	56.12	28.19	10.78	22.44
1	22	87.39	54.47	47.99	34.00	6.78	54.81	36.87	7.13	41.96
2	22	90.31	60.29	50.67	31.14	10.57	55.76	31.32	10.04	24.66
1	23	80.72	48.89	43.35	33.87	4.89	53.30	40.50	6.14	48.34
2	23	89.31	58.59	49.48	31.13	9.91	55.78	33.67	10.03	24.73
1	24	85.38	53.77	47.12	31.71	5.70	55.04	37.63	7.45	42.91
2	24	89.96	59.24	49.61	31.99	10.19	55.07	31.68	10.45	23.58
1	25	86.65	54.29	48.41	33.32	5.87	55.60	36.50	6.94	39.79
2	25	89.36	58.17	48.93	32.81	9.29	54.88	31.54	9.89	27.51
1	26	86.16	53.43	47.42	32.34	6.14	55.10	37.93	6.83	42.20
2	26	89.68	59.36	49.95	31.35	10.62	55.52	30.47	10.00	23.19

1	27	88.49	55.52	49.60	33.01	6.89	55.81	36.19	7.22	40.37
2	27	89.82	59.08	50.08	31.33	11.03	55.86	31.07	10.26	27.04
1	28	89.64	55.61	49.39	34.42	6.25	55.09	37.81	7.24	40.32
2	28	89.86	59.80	50.00	31.83	10.44	55.65	31.32	10.30	25.21
1	29	90.85	56.89	50.67	33.97	6.28	55.37	37.34	7.12	40.14
2	29	89.78	57.78	49.13	33.48	9.54	54.38	34.13	9.33	28.83
1	30	86.24	53.51	47.05	32.74	6.74	54.88	37.33	7.28	42.17
2	30	90.76	60.79	51.45	31.16	10.96	56.69	29.82	9.92	25.05
1	31	89.77	55.82	48.23	34.50	7.48	53.99	37.11	8.34	37.78
2	31	91.17	60.67	50.66	31.67	9.70	55.46	31.94	10.37	22.75
1	32	89.31	55.94	50.21	33.41	6.37	55.79	36.13	6.83	40.62
2	32	89.62	58.88	49.94	31.98	9.93	55.58	31.36	10.00	26.28
1	33	87.82	55.97	48.87	32.30	6.82	55.60	36.71	7.92	37.90
2	33	89.57	60.26	49.85	30.94	11.22	55.64	29.05	11.16	21.05
1	34	89.02	53.84	47.60	36.22	5.92	52.86	38.79	6.70	40.78
2	34	90.21	57.61	48.89	34.32	8.85	54.00	33.88	8.85	27.55
1	35	86.05	53.88	47.31	32.07	6.69	54.98	37.44	7.87	41.15
2	35	89.75	60.13	49.98	30.64	10.34	55.77	30.65	10.75	22.98
1	36	87.35	54.33	48.41	33.91	5.64	55.34	38.21	6.76	42.32
2	36	89.42	59.30	49.72	32.35	9.92	55.48	31.67	10.12	26.78
1	38	*	*	*	*	*	*	*	*	*
2	38	88.47	55.39	46.58	34.72	10.51	52.35	32.26	9.58	28.21
1	39	85.45	53.56	47.37	33.05	5.81	55.11	37.89	7.03	42.31
2	39	89.21	59.24	49.58	31.50	9.52	55.66	30.90	9.94	24.50
1	40	89.03	56.20	49.42	32.33	7.67	55.34	35.94	7.77	38.38
2	40	89.55	59.89	49.51	30.13	11.67	55.51	30.56	11.47	23.58
1	41	86.92	52.93	47.05	34.20	6.24	53.94	38.81	6.68	43.37
2	41	89.76	58.47	49.35	32.79	10.62	54.73	31.37	9.71	26.21
1	42	88.95	55.14	48.35	33.81	6.60	53.77	37.91	7.83	40.98
2	42	89.52	58.73	48.47	31.38	10.41	54.16	32.98	10.71	27.05
1	43	84.99	51.74	44.56	34.65	6.25	52.70	40.35	7.70	40.58
2	43	89.25	58.47	48.74	31.46	11.09	54.68	30.89	10.71	23.09
1	44	88.43	54.88	48.33	34.10	6.20	54.74	37.96	7.04	41.44
2	44	89.09	58.70	49.00	31.72	10.60	54.82	31.02	10.29	24.36
1	45	85.32	53.75	47.95	31.98	5.50	55.96	38.47	6.79	45.19
2	45	89.12	59.69	50.43	30.43	10.34	56.72	30.65	10.32	27.15
1	46	87.38	53.84	47.18	33.41	5.66	53.99	38.46	7.16	40.41
2	46	89.97	59.50	48.86	31.39	9.91	54.38	32.61	10.79	24.21
1	47	89.30	55.00	48.10	34.11	6.81	53.74	38.83	8.17	38.97
2	47	90.30	60.54	50.08	30.85	11.83	55.48	30.25	11.40	21.96
1	48	87.33	54.22	47.74	33.10	6.77	54.89	38.16	7.48	40.22
2	48	90.81	60.71	51.09	31.32	10.83	56.21	30.90	10.47	22.03
1	49	87.12	54.09	47.49	32.99	6.71	55.04	38.00	7.41	40.55
2	49	88.95	58.74	49.13	31.76	9.92	55.37	32.13	10.18	25.17
1	50	90.71	55.73	48.46	35.85	7.43	53.33	36.94	7.95	35.54
2	50	89.54	57.73	48.23	33.74	10.12	53.15	33.21	10.19	24.94
1	51	87.70	54.57	48.50	33.70	6.40	55.28	37.70	6.37	42.33
2	51	91.40	61.25	52.19	30.53	9.72	57.65	30.95	9.42	24.16
1	52	88.69	53.09	47.00	36.06	6.27	52.46	39.71	7.57	39.45
2	52	90.44	59.83	50.94	29.96	9.84	56.35	31.92	9.47	26.07

1	53	87.23	51.39	46.21	36.22	5.19	52.56	41.26	6.16	44.33
2	53	90.46	58.36	49.43	34.04	8.79	54.21	33.31	9.19	26.84
1	54	86.08	53.67	46.85	31.34	7.07	54.71	36.92	7.95	39.28
2	54	89.85	59.75	49.78	30.41	11.16	55.78	30.20	11.07	22.07
1	55	90.20	55.44	48.45	34.65	6.77	53.54	39.64	7.55	39.22
2	55	89.94	58.44	48.67	31.57	9.04	54.02	35.80	10.06	26.13
1	56	86.34	52.17	46.33	35.25	5.84	53.65	38.47	6.90	39.33
2	56	90.36	58.45	49.33	33.40	9.51	54.56	32.83	9.58	26.17
1	57	87.74	55.48	48.15	32.54	7.47	55.08	36.60	8.07	38.14
2	57	90.60	61.50	50.63	30.08	10.75	56.19	30.45	10.95	21.59
1	58	88.88	54.16	47.44	34.69	7.25	52.74	38.71	7.74	39.27
2	58	89.99	57.80	49.50	33.68	10.53	54.40	32.82	9.26	25.86
1	59	88.79	55.58	48.27	32.53	7.75	54.51	36.13	8.16	38.32
2	59	90.85	61.29	50.64	29.97	11.55	55.75	30.22	11.17	20.28
1	60	87.37	55.04	47.92	32.85	7.06	54.68	35.88	7.55	40.12
2	60	89.49	60.01	49.49	30.98	11.49	55.41	29.14	10.72	22.85
1	61	88.73	54.53	48.37	34.10	6.58	54.42	37.97	7.53	40.26
2	61	89.98	59.95	50.22	31.67	10.38	55.98	30.79	10.50	23.92
1	62	85.69	52.40	47.16	32.29	5.14	54.55	39.38	6.14	44.05
2	62	89.40	58.88	49.88	31.45	10.73	55.89	30.25	9.94	27.67
1	63	86.97	52.60	47.18	34.59	5.72	54.18	38.83	6.44	40.97
2	63	90.78	60.57	50.89	31.22	10.20	56.33	30.14	9.92	24.48
1	64	87.68	55.13	48.74	32.63	6.45	55.48	37.09	7.21	41.27
2	64	90.47	60.06	50.33	31.21	10.57	56.06	30.43	10.60	24.10
1	65	87.02	54.05	48.27	33.02	5.79	55.34	37.55	6.76	43.11
2	65	90.17	60.48	50.52	31.62	10.43	56.09	30.66	10.47	22.75
1	66	88.44	54.20	47.43	34.02	6.44	53.46	38.42	7.69	40.81
2	66	89.88	58.80	49.31	31.72	10.03	54.87	32.59	10.27	25.81
1	67	87.94	55.84	49.47	31.83	6.67	56.27	35.02	7.03	39.60
2	67	90.41	61.07	51.16	30.43	10.24	56.57	29.90	10.46	21.87
1	68	90.19	56.59	49.36	34.11	7.58	54.36	36.83	8.41	36.50
2	68	90.26	59.78	49.39	31.45	10.48	54.70	33.57	10.83	22.32
1	69	85.82	53.30	47.38	33.19	6.31	55.31	37.93	7.01	41.42
2	69	90.42	60.43	51.14	31.37	10.64	56.60	29.85	9.66	23.57
1	70	88.89	57.72	50.91	30.72	7.65	57.13	35.23	7.88	38.09
2	70	89.53	60.56	50.36	29.78	11.42	56.43	29.51	11.10	23.42
1	71	87.88	54.83	48.37	32.81	5.99	54.96	37.44	7.05	41.49
2	71	90.03	59.29	50.00	31.48	9.03	55.61	32.56	9.90	26.09
1	72	90.37	55.85	48.95	35.05	6.46	53.89	37.64	7.25	38.49
2	72	90.33	59.57	49.73	32.27	10.46	54.79	31.44	9.83	22.63
1	73	86.86	53.24	46.84	33.13	6.57	53.79	37.68	7.26	42.19
2	73	89.84	59.94	50.11	31.05	10.72	55.64	30.07	10.56	23.39
1	74	87.39	52.70	47.36	35.26	5.58	54.16	39.64	6.68	42.65
2	74	90.25	58.51	50.26	33.21	9.77	55.91	31.89	9.48	26.12
1	75	90.56	56.50	49.29	34.54	7.11	53.92	36.82	7.50	37.83
2	75	91.59	60.93	50.47	32.10	10.37	55.12	31.51	10.41	22.05
1	76	88.96	54.28	47.81	35.47	5.61	53.37	38.76	7.01	40.20
2	76	91.00	60.06	50.23	32.06	9.78	55.27	32.26	9.99	24.65
1	77	88.06	55.41	48.89	32.92	6.72	55.35	36.79	7.15	40.98
2	77	89.56	59.75	49.79	31.66	10.09	55.91	30.69	10.36	25.02

1	78	89.29	56.61	49.66	33.45	6.55	55.44	37.15	7.86	40.45
2	78	89.98	59.71	50.18	31.98	9.50	55.68	33.08	9.61	26.68
1	79	87.52	56.02	49.14	30.94	7.15	55.93	35.31	8.19	37.65
2	79	88.86	58.65	48.70	30.63	10.29	55.42	30.91	10.64	26.98
1	80	89.58	56.49	50.02	32.51	6.28	55.84	37.06	7.10	40.03
2	80	90.31	61.01	51.22	30.10	10.56	56.58	30.30	10.25	23.18
1	81	88.67	52.68	46.63	36.24	6.15	52.27	40.57	7.51	39.42
2	81	89.73	57.73	47.34	32.96	10.07	52.51	34.28	10.63	24.17
1	82	87.41	53.83	48.36	33.61	5.40	55.27	39.73	6.26	43.51
2	82	90.05	59.77	50.11	30.83	9.31	55.74	32.32	9.85	26.07
1	83	88.61	54.09	47.81	34.64	6.70	53.93	37.92	7.33	40.38
2	83	90.27	59.65	50.03	32.13	9.86	55.25	32.26	10.35	23.20
1	84	86.23	53.32	46.99	32.49	6.53	54.61	38.04	7.41	40.43
2	84	89.42	59.21	49.66	31.11	10.50	55.60	31.47	10.30	24.10
1	85	88.07	55.59	49.30	32.25	6.21	55.95	37.24	6.93	42.40
2	85	89.93	60.60	50.58	31.17	10.89	56.37	29.06	10.41	24.77
1	86	86.99	54.78	48.18	32.14	6.67	55.69	37.94	7.55	40.17
2	86	88.12	59.19	48.99	30.05	10.14	55.52	31.33	10.79	23.75
1	87	85.57	52.39	46.22	33.79	6.00	54.06	38.86	7.21	42.70
2	87	89.18	60.15	49.44	30.72	10.82	55.69	29.50	11.03	21.52
1	88	88.96	55.64	49.55	32.73	6.10	55.22	37.73	7.19	42.19
2	88	90.71	59.90	50.53	31.50	9.56	55.64	32.71	9.88	25.58
1	89	90.00	53.93	48.09	36.20	6.00	52.95	39.37	6.81	40.46
2	89	90.31	57.69	48.40	34.43	10.33	53.20	32.31	9.85	24.29
1	90	90.59	57.38	50.34	33.02	6.98	55.33	36.81	7.59	39.69
2	90	89.40	59.85	49.36	30.75	11.22	55.04	31.36	10.90	23.37
1	91	89.33	54.39	48.21	35.16	5.64	53.84	38.50	6.74	41.80
2	91	90.34	57.83	48.44	33.87	9.80	53.61	33.34	9.84	27.26
1	92	87.22	53.57	47.65	33.90	5.84	54.59	39.07	6.78	41.28
2	92	90.20	60.25	50.67	31.28	10.24	55.89	30.64	9.90	22.03
1	93	88.82	56.27	50.10	32.91	6.67	56.24	36.30	7.19	41.13
2	93	90.52	60.55	51.21	31.50	10.42	56.51	29.91	9.97	24.02
1	94	90.40	56.78	50.74	32.79	6.55	56.11	36.98	6.99	39.42
2	94	90.43	61.25	51.37	29.97	9.77	56.69	31.13	10.46	22.95
1	95	89.99	55.32	49.20	34.95	6.20	54.35	38.70	7.30	39.00
2	95	91.04	60.25	51.17	31.91	10.92	56.50	32.52	9.72	22.88
1	96	87.31	54.31	48.54	33.35	5.90	55.18	37.10	7.11	43.23
2	96	89.97	58.56	50.32	31.78	8.81	56.30	34.00	9.77	27.93
1	97	88.55	55.16	49.57	34.15	5.69	55.80	37.56	6.14	41.78
2	97	90.25	60.40	51.52	31.90	8.91	56.95	32.81	8.89	27.15
1	98	88.68	54.41	47.18	33.58	6.99	53.33	38.07	8.13	38.37
2	98	90.56	59.35	48.93	31.66	10.61	53.93	32.22	11.01	20.00
1	99	87.09	53.76	47.63	33.14	5.74	54.70	39.02	6.81	44.00
2	99	88.96	58.63	49.12	31.50	10.49	55.34	31.64	10.61	26.20
1	100	85.55	51.08	46.07	34.86	4.87	54.01	40.97	5.89	46.87
2	100	88.80	58.62	49.08	32.08	9.54	55.62	31.40	9.78	26.67
1	101	88.54	56.34	50.09	31.96	7.43	56.78	35.48	7.57	39.90
2	101	89.48	60.65	50.72	30.60	11.00	56.73	29.34	10.72	21.49
1	102	85.76	52.17	46.15	34.49	5.98	53.41	39.20	6.99	42.58
2	102	89.83	59.28	49.66	31.66	10.04	54.88	31.49	10.03	23.10

1	103	87.06	52.52	46.58	34.17	6.90	53.31	38.91	7.37	41.44
2	103	89.23	58.36	48.28	31.81	11.23	53.91	32.04	11.06	23.58
1	104	88.39	54.45	49.55	33.40	5.02	56.00	38.18	5.68	44.05
2	104	89.86	59.56	50.82	32.00	9.71	56.43	31.06	9.38	26.37
1	105	89.33	55.88	48.97	34.38	6.63	54.59	36.90	7.49	39.54
2	105	90.34	60.22	50.11	31.13	10.79	55.57	31.27	10.63	24.32
1	106	90.97	56.91	50.04	34.39	6.70	54.53	37.42	7.89	37.90
2	106	90.44	59.26	50.45	32.47	9.38	55.41	34.08	9.83	27.57
1	107	87.84	56.31	49.72	31.27	6.85	56.52	35.93	7.46	40.68
2	107	89.73	61.22	50.64	29.54	10.91	56.56	29.81	11.05	23.37
1	108	90.10	57.28	51.20	33.07	6.81	56.90	35.22	7.27	39.89
2	108	89.94	60.39	50.37	31.80	10.95	55.53	28.27	10.70	21.77
1	109	87.72	55.03	48.19	33.65	6.78	54.81	36.18	7.73	38.64
2	109	89.62	59.05	48.83	32.38	11.12	54.90	30.13	10.86	23.78
1	110	87.90	56.01	49.95	32.58	6.98	56.55	36.33	7.30	40.42
2	110	89.98	60.54	50.97	30.40	10.84	56.75	30.17	10.45	25.38
1	111	89.05	56.13	48.84	33.31	7.78	54.83	35.48	8.04	39.02
2	111	89.77	59.57	49.55	31.02	10.42	55.04	32.54	10.62	23.73
1	112	88.26	54.65	49.20	33.55	6.16	55.83	38.41	7.14	40.16
2	112	89.99	59.85	50.36	31.51	10.43	56.35	31.59	10.54	24.09
1	113	87.55	54.47	48.47	31.07	5.25	55.21	37.97	6.67	40.70
2	113	89.95	61.64	50.50	28.95	11.90	56.68	27.79	11.71	21.64
1	114	87.76	54.39	48.55	33.92	6.48	54.92	37.97	6.97	41.12
2	114	89.38	59.36	49.74	31.41	9.48	55.41	32.87	10.04	26.64
1	115	89.54	57.43	49.21	32.72	8.45	55.15	34.33	9.04	35.57
2	115	89.62	60.96	49.30	30.36	11.76	55.38	28.82	12.59	21.17
1	116	87.50	54.27	48.27	34.07	5.97	55.12	36.77	6.62	42.08
2	116	90.01	60.32	50.35	31.97	10.61	55.71	29.70	10.44	23.03
1	117	89.72	57.43	51.73	32.08	6.16	57.58	35.82	6.46	40.48
2	117	91.55	63.25	53.65	29.63	10.79	58.66	27.62	10.00	22.58
1	118	87.55	55.61	49.22	32.04	6.18	55.99	36.25	7.02	40.76
2	118	90.12	61.45	51.49	29.67	10.79	57.16	28.42	10.65	21.96
1	119	87.90	55.30	49.08	32.68	6.73	55.76	36.41	7.26	41.51
2	119	89.78	60.26	50.54	31.02	10.99	56.50	28.84	10.76	24.99
1	120	86.83	52.40	47.02	34.91	5.17	54.13	38.75	6.43	42.72
2	120	90.38	58.49	49.45	32.64	8.71	54.76	34.63	9.82	24.78
1	121	88.07	55.02	49.31	33.95	5.62	55.95	37.55	6.41	40.25
2	121	89.68	59.74	50.09	31.25	9.02	55.85	32.11	9.87	26.50
1	122	88.09	54.85	48.67	33.14	6.56	55.08	38.15	7.35	41.14
2	122	90.00	60.33	50.17	30.13	10.21	55.92	32.26	10.72	25.36
1	123	88.07	54.86	48.01	33.86	6.90	53.99	36.54	7.57	38.82
2	123	90.26	58.81	49.28	32.54	10.07	55.10	34.76	10.03	26.47
1	124	86.45	53.87	47.23	33.00	6.44	54.89	37.97	7.35	41.92
2	124	90.22	60.28	50.39	30.99	10.32	56.04	30.85	10.02	23.81
1	125	87.78	55.94	49.29	31.98	6.54	56.16	37.16	7.20	42.03
2	125	90.87	61.72	51.91	30.32	10.97	57.19	30.27	10.37	24.00
1	126	87.00	53.24	46.94	33.93	6.16	53.85	39.30	7.11	41.75
2	126	90.17	60.46	50.07	30.57	10.40	55.41	32.07	10.78	22.11
1	127	88.73	55.20	49.55	34.06	5.24	55.59	38.69	6.49	43.07
2	127	89.87	58.22	49.31	32.96	8.58	54.82	34.54	9.55	29.60

1	128	87.74	54.74	48.28	33.17	6.73	54.91	36.98	7.45	41.07
2	128	89.99	60.15	50.16	31.67	10.86	55.76	29.29	10.58	22.52
1	129	88.85	56.55	50.24	32.70	6.48	56.59	35.81	6.98	38.27
2	129	89.87	61.16	50.62	30.62	11.00	56.61	28.86	10.72	23.21
1	130	86.84	54.38	48.00	33.41	5.49	55.03	37.35	6.71	42.96
2	130	89.58	60.47	50.19	30.80	9.33	55.81	30.51	10.23	22.54
1	131	89.86	57.21	50.96	32.70	7.00	56.46	36.95	7.72	38.60
2	131	90.44	61.01	50.89	30.47	10.99	56.44	30.29	11.04	22.69
1	132	88.94	52.51	47.14	36.47	4.87	52.33	40.98	6.06	41.63
2	132	90.16	56.88	48.05	33.97	8.61	53.03	34.99	9.29	27.10
1	133	86.85	53.34	48.18	33.33	5.08	55.32	38.69	6.44	43.59
2	133	89.71	60.28	50.67	29.69	9.77	56.42	30.93	10.44	25.35
1	134	86.14	52.87	46.78	34.18	5.18	54.04	39.41	6.50	41.44
2	134	90.15	59.16	49.63	32.92	10.25	54.98	29.78	9.93	24.28
1	135	88.77	54.15	48.52	35.11	4.61	52.84	39.89	5.99	39.15
2	135	90.28	58.24	50.12	32.97	8.92	55.30	32.64	9.00	27.78
1	136	86.54	54.01	48.28	32.90	5.59	55.17	38.74	6.70	43.18
2	136	90.22	60.12	50.73	31.04	9.95	56.13	31.13	10.38	24.34
1	137	89.94	55.38	49.92	34.06	6.12	55.18	38.12	6.25	41.58
2	137	89.80	58.71	49.71	32.47	9.67	55.03	32.37	9.18	24.51
1	138	87.43	55.41	49.16	31.89	6.63	56.31	36.81	7.17	40.32
2	138	89.81	61.00	51.79	30.30	10.32	57.92	29.15	9.84	24.57
1	139	87.25	53.69	48.50	33.36	5.63	55.50	37.99	6.24	43.91
2	139	89.67	59.57	50.05	30.92	10.19	55.85	31.53	10.45	25.34
1	140	88.51	56.16	49.02	33.13	7.19	55.19	35.42	8.19	38.78
2	140	89.69	59.73	49.51	31.70	10.82	55.22	30.96	11.30	26.03
1	141	89.58	55.56	50.38	34.00	5.75	55.82	37.54	5.98	42.43
2	141	90.76	59.75	51.37	31.83	9.45	56.55	33.16	9.15	25.65
1	142	91.42	60.25	52.55	30.86	7.78	57.66	32.81	8.18	34.92
2	142	90.58	61.29	51.00	30.54	11.32	56.71	29.25	10.99	21.96
1	143	88.89	56.48	50.44	32.69	5.50	56.52	36.69	6.60	42.19
2	143	90.20	60.99	51.72	30.80	11.10	57.26	28.17	10.15	23.34
1	144	85.04	50.63	44.66	34.74	6.17	52.57	40.20	7.05	44.10
2	144	89.54	58.95	49.32	31.50	9.68	55.21	32.41	10.01	25.25
1	145	89.78	54.86	48.36	35.27	6.53	53.43	39.34	7.53	40.25
2	145	90.24	58.96	49.35	31.82	10.39	54.78	33.45	10.33	24.29
1	146	86.29	53.15	47.18	33.15	6.19	54.62	37.87	7.06	43.53
2	146	89.18	58.76	48.80	31.10	10.86	54.90	31.74	10.53	26.76
1	147	87.79	54.65	48.13	33.79	5.91	55.06	37.62	7.04	41.41
2	147	89.71	60.38	50.01	30.93	10.78	56.13	29.82	10.88	21.81
1	148	89.35	54.39	48.05	35.46	6.26	53.75	38.10	7.21	40.73
2	148	90.62	59.03	49.48	32.93	9.57	54.53	32.83	9.79	26.80
1	149	88.07	54.87	49.88	32.17	5.38	56.33	38.11	5.70	45.03
2	149	90.56	60.06	51.60	31.09	9.92	57.31	30.84	9.42	26.09
1	150	88.55	55.50	48.14	32.73	7.46	54.37	36.65	8.02	38.07
2	150	90.91	61.07	50.18	30.36	11.24	55.52	30.92	11.45	20.20
1	151	87.38	54.05	47.96	33.31	5.79	54.82	38.48	6.81	40.22
2	151	89.76	59.54	49.94	31.03	9.87	55.54	31.32	10.05	24.00
1	152	87.61	54.99	48.64	33.00	6.05	55.24	37.05	6.93	40.07
2	152	88.76	58.28	48.53	31.10	10.50	54.71	31.02	10.43	25.04

1	154	86.92	53.68	48.75	32.90	5.42	56.05	37.87	5.63	46.28
2	154	90.94	60.28	51.50	31.69	9.41	56.70	31.32	9.04	26.84
1	155	86.51	53.18	46.39	33.33	5.86	53.65	38.64	7.23	41.85
2	155	90.14	59.93	49.89	31.00	10.16	55.34	30.75	10.50	23.65
1	156	87.27	54.81	49.38	32.10	5.79	56.39	37.26	6.27	43.84
2	156	89.47	58.30	49.82	32.53	8.28	55.82	33.90	8.87	30.05
1	158	89.49	55.63	48.40	34.24	6.14	54.19	38.39	7.35	40.34
2	158	90.74	60.17	50.59	31.39	9.29	56.05	32.27	10.21	24.47
1	160	87.30	53.37	47.20	33.75	5.87	53.93	38.83	7.04	41.94
2	160	88.90	58.38	48.26	31.55	10.11	54.42	32.22	10.77	24.99
1	161	88.37	54.96	49.02	33.94	5.33	55.35	37.47	6.39	43.55
2	161	90.07	59.35	50.22	31.92	9.68	55.63	30.94	9.73	25.62
1	162	86.15	52.93	46.44	33.57	6.70	53.79	38.13	7.89	40.15
2	162	88.91	57.83	47.80	30.90	10.24	53.87	33.50	10.93	23.88
1	163	88.40	55.12	48.54	33.84	7.05	54.69	37.34	7.65	39.12
2	163	89.67	59.77	49.45	30.45	10.54	55.51	31.45	11.04	26.10
1	164	89.46	56.55	51.04	33.22	5.28	56.89	36.52	6.35	43.93
2	164	90.89	61.07	51.49	32.57	9.53	56.48	29.77	10.11	24.38
1	165	86.98	52.90	46.52	34.09	6.56	53.67	38.98	7.36	42.59
2	165	89.83	59.71	50.10	31.53	9.84	55.49	31.36	10.45	23.77
1	166	88.16	56.51	50.41	31.54	6.41	56.98	36.18	7.08	42.30
2	166	90.92	61.46	51.92	30.95	10.24	57.38	29.48	10.26	24.67
1	168	90.90	58.06	50.58	33.17	7.16	55.78	35.21	7.84	38.61
2	168	89.42	59.78	49.79	30.49	10.91	55.39	31.54	10.71	23.91
1	169	89.75	54.13	47.48	36.15	6.77	52.46	39.23	7.51	40.12
2	169	90.15	58.38	49.02	33.51	9.83	54.03	32.72	9.65	26.49
1	170	86.19	54.25	47.57	32.22	6.39	55.28	37.17	7.18	41.51
2	170	89.93	60.71	51.34	29.86	10.23	57.23	30.91	10.13	23.55
1	172	86.80	54.10	48.43	32.65	5.28	55.66	37.98	6.28	42.21
2	172	89.34	59.23	49.78	32.00	9.24	55.77	31.22	9.63	25.69
1	173	87.22	55.24	47.26	32.68	7.97	54.51	35.87	8.69	37.12
2	173	88.77	59.51	48.32	30.16	12.06	54.84	30.09	11.97	22.91
1	174	83.71	50.41	44.28	33.36	5.50	53.11	39.29	6.88	44.07
2	174	87.83	56.37	46.77	32.28	9.28	53.56	33.66	10.09	29.69
1	175	85.20	53.60	47.02	32.34	5.84	54.85	37.70	7.05	42.49
2	175	89.16	59.92	50.26	29.98	9.78	56.28	31.39	10.25	25.96
1	176	87.27	51.48	46.39	35.90	5.24	52.51	41.73	6.35	43.80
2	176	89.86	58.36	49.27	32.51	9.60	55.18	34.38	9.45	27.91
1	177	85.62	53.05	47.00	32.98	5.43	54.94	39.14	6.58	46.74
2	177	88.45	57.92	48.80	32.03	9.75	55.27	32.00	9.98	26.31
1	178	87.51	55.23	49.10	32.72	6.15	56.09	36.86	6.73	40.14
2	178	90.14	60.22	50.38	30.98	10.26	56.25	31.19	10.29	25.26
1	179	89.08	55.75	50.89	33.60	5.76	56.44	36.65	6.57	39.94
2	179	90.83	61.25	52.30	30.72	10.43	57.66	31.00	9.41	27.81
1	180	87.55	53.46	47.60	33.82	5.72	54.16	38.84	6.75	43.73
2	180	90.11	59.43	49.59	31.68	10.40	55.31	31.41	10.54	24.32
1	181	89.65	56.94	50.00	32.31	7.18	55.59	36.50	8.32	39.02
2	181	90.46	61.92	51.42	29.52	12.17	57.27	28.25	11.26	23.39
1	182	87.69	54.56	48.18	33.44	6.68	54.86	37.30	7.36	38.44
2	182	89.62	60.14	50.26	30.77	10.51	55.98	30.48	10.39	23.24

1	183	87.09	53.12	47.73	34.30	5.69	54.19	39.07	6.46	42.56
2	183	89.83	59.61	50.01	30.94	10.41	55.49	31.22	10.39	24.71
1	184	88.47	56.00	49.29	32.81	7.97	55.95	36.02	8.22	38.32
2	184	90.01	60.95	50.60	29.98	11.19	56.55	30.59	11.21	23.87
1	186	88.76	54.32	46.95	34.81	7.37	52.59	37.85	8.42	38.50
2	186	89.90	59.38	49.14	31.96	10.90	54.45	31.00	11.02	23.20
1	187	88.62	55.75	50.03	32.66	6.04	56.33	36.68	6.71	42.72
2	187	90.41	60.25	50.58	31.43	10.14	56.30	32.03	10.34	25.35
1	188	90.22	56.96	49.73	33.27	8.10	54.97	35.50	8.01	36.62
2	188	90.30	61.06	50.67	30.07	11.62	56.20	29.89	11.05	22.37
1	189	89.28	54.82	47.83	34.33	5.49	53.73	39.89	7.21	42.66
2	189	89.25	58.82	47.77	31.18	11.43	53.80	34.57	11.76	22.56
1	190	88.63	55.06	49.13	33.02	6.81	55.23	37.27	7.21	41.19
2	190	90.69	60.08	50.63	31.57	10.06	55.89	31.62	10.34	23.56
1	191	86.14	53.90	48.03	32.62	6.14	55.78	37.62	6.82	43.38
2	191	90.03	61.03	50.66	30.20	10.98	56.28	30.50	10.80	24.30
1	192	88.89	55.48	49.08	33.79	6.46	55.22	36.26	7.47	40.15
2	192	90.32	60.04	50.32	30.86	10.03	56.13	30.87	10.38	23.91
1	193	88.42	57.08	48.36	31.55	8.46	54.97	34.53	9.49	37.81
2	193	87.47	59.94	48.64	26.90	13.23	56.64	29.38	12.31	22.30
1	194	85.94	53.24	47.32	32.67	5.85	54.71	38.16	6.96	42.17
2	194	89.09	59.18	49.63	31.62	10.22	55.78	30.90	10.22	26.20
1	195	85.22	49.40	45.27	36.39	4.00	52.36	41.92	5.18	44.09
2	195	89.90	58.44	49.64	33.10	10.56	55.45	32.06	9.52	25.93
1	196	88.36	56.02	48.87	32.08	7.98	55.19	35.76	8.27	38.18
2	196	89.52	60.12	49.50	29.96	11.19	54.95	31.42	11.33	24.01
1	197	87.50	53.03	46.64	34.89	6.08	53.03	39.87	7.11	41.59
2	197	89.35	57.25	47.50	33.22	9.55	52.85	34.87	10.14	26.54
1	199	87.37	55.50	49.76	31.74	6.31	56.57	36.72	6.82	42.89
2	199	89.52	60.75	50.78	30.24	10.59	56.73	29.67	10.61	24.84
1	200	87.25	52.77	46.63	34.31	5.99	53.42	39.42	6.92	42.11
2	200	89.99	58.96	49.44	32.72	10.10	55.17	32.20	9.88	25.98