Supplementary Material

Cell wall composition and biomass saccharification potential of Sida hermaphrodita differ between genetically distant accessions

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**Table S1. Sida accessions from 16 different origins, their identities in the phylogenetic tree, their geographical origins and their contribution to phylogenetic analyses.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| No | Identity | Identity (phyl. tree) | Origin | Country of origin | No. plants analysed by GBS | No. plants after GBS quality control | Provider / Accession number |
| 1 | SH1\_P | 1P... | natural habitat, Kentucky | USA | 18 | 17 | Christian Wever / CW2016131 |
| 2 | SH2\_P | 2P... | natural habitat, West Virginia | USA | 16 | 16 | Christian Wever / CW2016132 |
| 3 | SH3\_P | 3P... | natural habitat, West Virginia | USA | 18 | 17 | Christian Wever / CW2016135 |
| 4 | SH\_H | H... | Botanical Garden Hohenheim | Germany | 13 | 10 | XX-0-HOH-SYS-3993 |
| 5 | SH\_L | L... | Botanical Garden Leipzig | Germany | 12 | 12 | XX-0-LZ-WNA-7-2013 |
| 6 | SH\_UF | UF... | variety Fitoenergia | Ukraine | 13 | 8 | Plant Production Institute nd. A. V.Ya.Yuryev of the National Academy of Agrarian Science of Ukraine, Kharkiv |
| 7 | SH\_UV | UV... | variety Virdjinia | Ukraine | 7 | 6 | Plant Production Institute nd. A. V.Ya.Yuryev of the National Academy of Agrarian Science of Ukraine, Kharkiv |
| 8 | SH\_J | J... | Jelitto company | Germany | 13 | 13 | Jelitto perennial seeds |
| 9 | SH\_Jü | Ju... S... | Forschungzentrum Jülich | Poland / Germany | 13 | 13 | Forschungzentrum Jülich, Field Daubenrath 2014 |
| 10 | SH\_A | A... | Forschungzentrum Jülich | Austria | 13 | 13 | Forschungzentrum Jülich, N.D. Jablonowski |
| 11 | SH\_Hu | Hu... | University of Debrecen | Hungary | 13 | 13 | Erika Kurucz (selection of Dr. Zoltán Kováts) |
| 12 | SH\_HUS | US... | University of Debrecen | USA / Hungary | 13 | 13 | Erika Kurucz (USA, natural population 2009) |
| 13 | SH\_K | K... | BG KIT, Karlsruhe | Germany | 12 | 11 | - |
| 14 | SH\_P | P... | University of Warmia and Mazury in Olsztyn | Poland | 13 | 8 | Jacek Kwiatkowski |
| 15 | SH\_R | R... | University of Târgu Mureș | Romania | 2 | 1 | - |
| 16 | SH\_D | D... | Botanical Garden Düsseldorf | Germany | 3 | 3 | - |
| Total |  |  |  |  | 192 | 174 |  |

**Table S2. Mean values of Cell wall composition (chemotype) of the seven different Sida accessions.** Mean values of and standard deviation (stdev.) of crystalline cellulose (CrC), acetyl groups, acetyl bromide lignin (ABSL) and polysaccharides in TFA fraction, in % of the total TFA fraction; Leipzig (L): n=5; Hohenheim (H): n=6; Jülich (Jül): n=3; Karlsruhe (K): n=4; Kentucky (SH1): n=15; West Virginia location 1 (SH2): n=4; West Virginia location 2 (SH3): n=4.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Accession |  | Acetyl groups | CrC | ABSL | Hemicellulose (total TFA) | Rhamnose | Arabinose | Galactose | Glucose | Xylose | Mannose | Gal. Acid | 72h Accellerase |
|  |  | wt% / dAIR | | | | µg / mg dAIR | | | | | | | wt% Glc / Glucan |
| L | mean | 2.92 | 33.88 | 15.54 | 18.03 | 2.40 | 4.25 | 6.47 | 5.18 | 38.33 | 1.79 | 9.18 | 29.39 |
| stdev. | 0.49 | 2.78 | 0.97 | 1.49 | 0.86 | 0.72 | 1.18 | 0.75 | 2.09 | 0.35 | 1.22 | 12.48 |
| H | mean | 3.71 | 31.16 | 19.75 | 15.00 | 1.10 | 2.99 | 4.04 | 3.10 | 36.51 | 1.45 | 7.06 | 31.09 |
| stdev. | 0.99 | 4.44 | 1.11 | 2.46 | 0.38 | 0.32 | 0.39 | 0.90 | 7.36 | 0.42 | 0.48 | 8.56 |
| Jül | mean | 3.98 | 34.73 | 17.94 | 18.26 | 2.29 | 4.95 | 6.09 | 3.99 | 40.78 | 1.95 | 8.42 | 26.01 |
| stdev. | 1.06 | 5.36 | 0.60 | 1.81 | 0.79 | 0.65 | 1.58 | 0.71 | 1.98 | 0.96 | 1.00 | 13.42 |
| K | mean | 5.47 | 41.35 | 19.03 | 19.17 | 2.33 | 4.47 | 5.52 | 3.13 | 43.87 | 1.83 | 10.74 | 32.60 |
| stdev. | 0.00 | 2.08 | 0.96 | 2.16 | 1.02 | 1.32 | 0.73 | 1.17 | 4.14 | 1.00 | 1.60 | 12.94 |
| SH1 | mean | 5.15 | 41.03 | 17.37 | 19.56 | 3.30 | 5.28 | 7.71 | 5.14 | 56.11 | 1.45 | 9.12 | 19.34 |
| stdev. | 0.65 | 5.72 | 3.17 | 2.53 | 1.26 | 1.46 | 2.03 | 1.99 | 11.18 | 1.09 | 5.14 | 4.62 |
| SH2 | mean | 4.72 | 38.53 | 18.18 | 19.35 | 1.78 | 4.37 | 6.08 | 2.74 | 46.19 | 1.37 | 10.01 | 34.39 |
| stdev. | 0.53 | 8.98 | 0.46 | 1.91 | 0.58 | 0.38 | 0.46 | 0.88 | 5.52 | 1.36 | 0.79 | 10.26 |
| SH3 | mean | 5.46 | 48.33 | 21.13 | 20.28 | 2.15 | 4.60 | 5.48 | 3.51 | 50.09 | 2.83 | 11.15 | 17.44 |
| stdev. | 0.46 | 3.12 | 1.62 | 1.33 | 1.09 | 0.83 | 0.53 | 1.51 | 3.81 | 1.22 | 1.76 | 8.88 |

**Table S3. Normalized data of cell wall characterization of untreated biomass for construction of PCA in SPSS.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Accession | Acetyl groups | CrC | ABSL | Hemicellulose (total TFA) | Rhamnose | Arabinose | Galactose | Glucose | Xylose | Mannose | Gal. Acid |
|  | wt%/ dAIR | | | | µg/mg dAIR | | | | | | |
| L | 0 | 0.26 | 0 | 0.86 | 0.58 | 0.22 | 0.52 | 1 | 0.03 | 0.42 | 0.3 |
| H | 0.28 | 0 | 0.55 | 0 | 0 | 0.54 | 0.13 | 0.1 | 0 | 0 | 0 |
| Jül | 0.42 | 0.13 | 0.43 | 0.04 | 0.53 | 0.73 | 0.36 | 0.51 | 0.17 | 0.51 | 0.03 |
| K | 0.91 | 0.64 | 0.63 | 1 | 0.49 | 0 | 0 | 0.12 | 0.36 | 0.44 | 0.71 |
| SH1 | 0.88 | 0.63 | 0.33 | 0.22 | 1 | 1 | 1 | 0.98 | 1 | 0.33 | 0.34 |
| SH2 | 0.71 | 0.5 | 0.47 | 0.9 | 0.28 | 0.31 | 0.36 | 0 | 0.48 | 0.21 | 0.59 |
| SH3 | 1 | 1 | 1 | 0.25 | 0.52 | 0.47 | 0.11 | 0.42 | 0.7 | 1 | 1 |

**Table S4. Cell wall composition (chemotype) of 41 clones of the seven different Sida accessions.** Mean values of the individual clones. Crystalline cellulose (CrC), acetyl groups, acetyl bromide lignin (ABSL) and polysaccharides in TFA fraction, in % of the total TFA fraction;

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No | Accession | Acetyl groups | CrC | ABSL | Hemicellulose (total TFA) | Rhamnose | Arabinose | Galactose | Glucose | Xylose | Mannose | Gal. Acid | 72h Accellerase |
|  |  | wt%/ dAIR | | | | µg/mg dAIR | | | | | | | wt% Glc/ Glucan |
| 1 | L | 2.52 | 37.61 | 14.39 | 17.18 | 1.10 | 4.43 | 5.46 | 5.95 | 37.51 | 1.93 | 8.04 | 20.60 |
| 2 | L | 2.55 | 30.11 | 14.69 | 19.79 | 3.28 | 4.95 | 8.01 | 5.68 | 40.30 | 1.51 | 10.46 | 18.97 |
| 3 | L | 2.81 | 33.35 | 16.24 | 19.48 | 2.90 | 4.79 | 7.23 | 5.52 | 40.39 | 1.80 | 10.43 | 28.70 |
| 4 | L | 3.00 | 33.07 | 16.61 | 17.10 | 2.69 | 3.88 | 6.44 | 4.46 | 35.40 | 2.30 | 8.97 | 28.45 |
| 5 | L | 3.73 | 35.25 | 15.75 | 16.58 | 2.01 | 3.18 | 5.21 | 4.31 | 38.06 | 1.40 | 8.01 | 50.26 |
| 6 | H | 2.74 | 28.24 | 19.01 | 14.71 | 0.85 | 3.14 | 3.96 | 2.76 | 35.96 | 1.52 | 6.97 | 31.38 |
| 7 | H | 5.22 | 39.00 | 18.57 | 13.63 | 1.32 | 2.58 | 4.14 | 2.79 | 32.28 | 0.99 | 7.02 | 24.35 |
| 8 | H | 3.42 | 27.43 | 20.04 | 15.86 | 0.81 | 3.19 | 3.63 | 2.92 | 40.21 | 1.58 | 7.13 | 46.65 |
| 9 | H | 4.63 | 33.06 | 20.61 | 16.16 | 1.71 | 2.95 | 4.69 | 3.53 | 38.15 | 2.04 | 7.51 | 25.69 |
| 10 | H | 2.89 | 31.37 | 21.37 | 18.45 | 0.71 | 3.41 | 4.18 | 4.63 | 47.08 | 1.65 | 7.53 | 33.83 |
| 11 | H | 3.36 | 27.86 | 18.88 | 11.20 | 1.19 | 2.68 | 3.65 | 1.98 | 25.34 | 0.95 | 6.22 | 24.62 |
| 12 | Jül | 3.34 | 34.91 | 17.30 | 20.35 | 3.12 | 5.68 | 7.86 | 4.81 | 42.75 | 3.05 | 9.05 | 23.86 |
| 13 | Jül | 5.20 | 40.01 | 18.50 | 17.26 | 2.21 | 4.41 | 5.61 | 3.55 | 38.78 | 1.24 | 8.94 | 40.37 |
| 14 | Jül | 3.39 | 29.28 | 18.01 | 17.17 | 1.54 | 4.76 | 4.81 | 3.62 | 40.81 | 1.56 | 7.26 | 13.80 |
| 15 | K | 5.47 | 35.27 | 18.01 | 16.08 | 2.16 | 3.95 | 5.44 | 2.05 | 35.82 | 0.87 | 10.01 | 35.38 |
| 16 | K | 5.47 | 45.89 | 20.49 | 22.00 | 3.46 | 4.29 | 5.25 | 4.25 | 51.71 | 2.04 | 11.50 | 17.86 |
| 17 | K | 5.47 | 43.17 | 19.33 | 18.56 | 1.01 | 3.59 | 4.98 | 2.78 | 45.02 | 2.70 | 9.52 | 31.78 |
| 18 | K | 5.47 | 41.06 | 18.30 | 20.05 | 2.69 | 6.07 | 6.41 | 3.45 | 42.92 | 1.72 | 11.92 | 45.37 |
| 19 | SH1 | 5.33 | 44.67 | 18.68 | 16.92 | 2.28 | 3.02 | 4.76 | 1.49 | 43.03 | 0.32 | 8.57 | 18.82 |
| 20 | SH1 | 4.58 | 44.59 | 21.06 | 23.31 | 1.94 | 3.40 | 7.45 | 3.63 | 57.17 | 1.53 | 12.28 | 12.40 |
| 21 | SH1 | 3.76 | 43.68 | 18.94 | 21.50 | 3.69 | 4.14 | 7.26 | 4.83 | 82.62 | 2.51 | 12.33 | 18.75 |
| 22 | SH1 | 5.60 | 41.90 | 13.79 | 23.22 | 5.22 | 6.41 | 9.53 | 7.74 | 68.83 | 2.86 | 17.08 | 16.32 |
| 23 | SH1 | 5.38 | 46.69 | 20.74 | 20.76 | 3.92 | 6.90 | 8.18 | 6.02 | 62.52 | 3.39 | 14.71 | 12.78 |
| 24 | SH1 | 4.79 | 32.70 | 15.27 | 21.88 | 2.40 | 5.61 | 6.60 | 3.90 | 50.50 | 0.88 | 12.15 | 29.56 |
| 25 | SH1 | 5.30 | 36.38 | 19.35 | 21.15 | 2.86 | 5.29 | 6.48 | 5.53 | 45.66 | 1.46 | 12.03 | 15.63 |
| 26 | SH1 | 5.43 | 44.94 | 16.52 | 18.23 | 2.84 | 3.71 | 4.85 | 4.10 | 69.28 | 2.33 | 12.07 | 15.59 |
| 27 | SH1 | 4.89 | 32.14 | 15.49 | 22.21 | 0.99 | 4.80 | 6.18 | 3.52 | 56.44 | 0.84 | 10.49 | 20.00 |
| 28 | SH1 | 5.35 | 42.96 | 23.15 | 18.99 | 2.35 | 4.21 | 6.70 | 4.64 | 56.46 | 1.12 | 12.38 | 18.31 |
| 29 | SH1 | 5.51 | 50.22 | 14.70 | 16.45 | 3.67 | 7.34 | 11.61 | 6.43 | 50.07 | 2.16 | 2.67 | 23.95 |
| 30 | SH1 | 5.67 | 43.90 | 13.81 | 17.15 | 5.30 | 5.96 | 8.80 | 8.34 | 55.61 | 2.21 | 2.82 | 22.18 |
| 31 | SH1 | 5.79 | 35.62 | 12.15 | 17.74 | 2.79 | 8.00 | 8.14 | 4.65 | 48.19 | 0.01 | 2.20 | 25.07 |
| 32 | SH1 | 3.90 | 32.20 | 17.09 | 16.97 | 4.72 | 5.22 | 7.63 | 3.59 | 40.05 | 0.21 | 2.23 | 19.94 |
| 33 | SH1 | 6.00 | 42.95 | 19.80 | 16.86 | 4.46 | 5.20 | 11.47 | 8.63 | 55.21 | 0.00 | 2.82 | 20.75 |
| 34 | SH2 | 5.20 | 35.78 | 18.41 | 17.32 | 1.19 | 3.96 | 5.97 | 2.13 | 42.57 | 0.19 | 8.92 | 33.37 |
| 35 | SH2 | 5.11 | 32.36 | 18.49 | 19.03 | 2.57 | 4.73 | 6.72 | 1.88 | 44.75 | 0.27 | 10.46 | 31.52 |
| 36 | SH2 | 4.10 | 51.83 | 18.32 | 21.94 | 1.72 | 4.14 | 5.99 | 3.26 | 54.36 | 2.08 | 10.70 | 24.12 |
| 37 | SH2 | 4.45 | 34.14 | 17.50 | 19.11 | 1.64 | 4.67 | 5.64 | 3.71 | 43.09 | 2.92 | 9.98 | 48.56 |
| 38 | SH3 | 5.88 | 53.00 | 20.32 | 19.37 | 3.04 | 4.52 | 5.14 | 5.41 | 53.10 | 4.56 | 11.94 | 10.59 |
| 39 | SH3 | 5.83 | 46.78 | 22.59 | 22.18 | 0.97 | 5.33 | 5.70 | 3.99 | 52.27 | 2.71 | 12.19 | 14.71 |
| 40 | SH3 | 5.15 | 46.44 | 22.36 | 20.26 | 3.12 | 5.09 | 6.13 | 2.64 | 44.65 | 2.35 | 11.97 | 30.49 |
| 41 | SH3 | 4.98 | 47.08 | 19.24 | 19.32 | 1.47 | 3.46 | 4.97 | 2.00 | 50.34 | 1.70 | 8.51 | 13.99 |

**Table S5. Pearson Correlation of Enzyamtic Saccharificaten (Glucose release / Glucan) with different cell wall features.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  | Acetyl groups | CrC | ABSL | Hemicellulose (total TFA) | Rhamnose | Arabinose | Galactose | Glucose | Xylose | Mannose | Gal. Acid |
|  | wt%/ dAIR | | | | µg/mg dAIR | | | | | | |
| all | -0.19 | -0.41 | -0.09 | -0.32 | -0.31 | -0.17 | -0.26 | -0.30 | -0.46 | -0.18 | -0.17 |

**Table S6. Scores of the first three Components for each accession**

|  |  |  |  |
| --- | --- | --- | --- |
| Accession | PC1 | PC2 | PC3 |
| L | -0.637 | 1.542 | -1.383 |
| Jul | -0.636 | 0.144 | 0.219 |
| H | -1.345 | -1.216 | 0.238 |
| K | 1.694 | -0.305 | -0.232 |
| SH1 | -0.011 | -0.725 | 0.143 |
| SH2 | 0.660 | -0.541 | -0.795 |
| SH3 | 0.275 | 1.101 | 1.810 |

**Table S7. Scores of the first three Components for each accession**

|  |  |  |  |
| --- | --- | --- | --- |
|  | PC1 | PC2 | PC3 |
| Acetyl groups | 0.841 | -0.276 | 0.399 |
| CrC | 0.984 | 0.044 | 0.065 |
| ABSL | 0.645 | -0.679 | 0.086 |
| Rha | 0.424 | 0.792 | 0.356 |
| Ara | -0.127 | 0.259 | 0.868 |
| Gal | -0.143 | 0.742 | 0.609 |
| Glc | -0.018 | 0.961 | 0.136 |
| Xyl | 0.714 | 0.187 | 0.645 |
| Man | 0.794 | 0.209 | -0.230 |
| GalA | 0.930 | -0.128 | -0.230 |
| Acetyl groups | 0.841 | -0.276 | 0.399 |

**Table S8. Wet-chemical composition of the pulp of Hohenheim and SH1 Accession after OrganoCat pretreatment.** Mean values and standard deviation of Crystalline cellulose (CrC), acetyl groups, acetyl bromide lignin (ABSL) and polysaccharides in TFA fraction, in % of the total TFA fraction; n =5.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | |  |  |  |  |  | |  | |  | |  |  |  | |  | |  | |  | |  |
| Accession | | conditions | Acetyl groups | | CrC | ABSL | Total TFA sugars | Pulp | Fuc | | Rha | | Ara | | Gal | Glc | Xyl | | Man | | Gal. Acid | | Glc. Acid | | Saccharification |
|  |  |  | [% sample biomass wt.] | | | | | | TFA fraction [% sample biomass wt.] | | | | | | | | | | | | | | | | (wt% Glc/ Glucan) |
| SH1 | mean | 125 °C, 3 h | 2.28 | 52.24 | | 21.97 | 13.81 | 53.2 | n.a. | 0.25 | | 0.01 | | 0.88 | | 2.14 | | 9.01 | | 0.77 | | 0.76 | | n.a. | 27.99 |
| stdev. | 0.10 | 4.83 | | 2.98 | 0.48 | 3.2 |  | 0.02 | | 0.00 | | 0.07 | | 0.18 | | 0.36 | | 0.03 | | 0.09 | |  | 5.02 |
| mean | 140 °C, 3 h | 0.72 | 69.74 | | 20.83 | 6.34 | 46.41 | n.a. | 0.07 | | n.a. | | 0.19 | | 2.78 | | 2.69 | | 0.42 | | 0.19 | | n.a. | 42.70 |
| stdev. | 0.05 | 5.72 | | 3.81 | 0.45 | 0.39 |  | 0.01 | | n.a. | | 0.02 | | 0.34 | | 0.10 | | 0.04 | | 0.02 | |  | 5.1 |
| H | mean | 125 °C, 3 h | 2.07 | 63.18 | | 21.53 | 14.01 | 57.49 | n.a. | 0.25 | | 0.03 | | 0.81 | | 2.27 | | 8.92 | | 0.83 | | 0.84 | | 0.06 | 17.53 |
| stdev. | 0.26 | 3.06 | | 1.57 | 0.50 | 0.92. |  | 0.01 | | 0.02 | | 0.04 | | 0.08 | | 0.47 | | 0.03 | | 0.05 | | 0.00 | 3.2 |
| mean | 140 °C, 3 h | 0.72 | 69.03 | | 19.99 | 5.36 | 49.17 | n.a. | 0.07 | | 0.01 | | 0.18 | | 2.21 | | 2.29 | | 0.43 | | 0.17 | | n.a. | 33.75 |
| stdev. | 0.15 | 4.29 | | 1.13 | 0.66 | 0.97 |  | 0.01 | | 0.00 | | 0.04 | | 0.35 | | 0.39 | | 0.02 | | 0.01 | |  | 4.5 |

**Table S9. Monosaccharide composition of the Hydrolysate fraction of Hohenheim and SH1 accession after OrganoCat pretreatment; n =3.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Accession | | conditions | Fucose | Rhamnose | Arabinose | Galactose | Glucose | Xylose | Mannose | Gal. Acid | Gluc. Acid |
|  |  |  | [wt% original biomass] | | | | | | | | |
| SH1 | mean | 125 °C, 3 h | 0.183 | 0.682 | 2.124 | 10.423 | 6.979 | 6.891 | 0.561 | 2.125 | 0.169 |
| stdev. | 0.019 | 0.096 | 0.183 | 0.607 | 0.493 | 0.649 | 0.090 | 0.205 | 0.015 |
| mean | 140 °C, 3 h | 0.186 | 1.988 | 1.992 | 13.056 | 10.613 | 13.559 | 1.343 | 3.319 | 0.455 |
| stdev. | 0.014 | 0.176 | 0.127 | 0.659 | 0.923 | 0.446 | 0.061 | 0.194 | 0.087 |
| H | mean | 125 °C, 3 h | 0.175 | 0.637 | 2.047 | 9.549 | 4.044 | 9.138 | 0.763 | 1.869 | 0.132 |
| stdev. | 0.009 | 0.048 | 0.087 | 0.684 | 0.284 | 0.508 | 0.050 | 0.097 | 0.006 |
| mean | 140 °C, 3 h | 0.198 | 1.836 | 2.091 | 12.398 | 7.089 | 15.637 | 1.753 | 3.027 | 0.414 |
| stdev. | 0.015 | 0.109 | 0.108 | 1.092 | 0.652 | 1.075 | 0.115 | 0.234 | 0.021 |