Breeding for biomass sorghum in Europe: a global review on target traits and plant ideotypes depending on cropping conditions and transformation process.

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Economic and Political Context for crop biomass production in Europe

✓ International demand in energy will increase of 50% before 2030
✓ Fossil resources are limited (40-60 years for oil, 70 years of natural gaz, 200 years of charcoal
✓ Global warming

Targets 2020 for Europe:
✓ 20% of energy and 10% of transport fuels from renewable origin
⇒ Future large areas for crops dedicated to energy production (e.g. France: to 1.2 millions ha)

Sustainable fuel production has to be based on lignocellulose products and by-products
Routes for transformation of lignocellulosic biomass to biofuels and/or energy

Biomass

Thermochemical route
- Gasification
  - Syngas
    - Fischer-Tropsch synthesis
      - Heat
      - Power
    - BTL
      - DME, methanol
        - DME, methanol
      - Hydrogen
        - Fuel cells
      - Transport fuels
        - BTL
          - Methanol
            - Methane
              - Power
            - Ethanol
              - Methanisation
                - Biogas
  - Pyrolysis/Liquefaction
    - Pyrolytic oil
    - Pretreatment
      - Hydrolysate
        - Fermentation
          - Ethanol
            - Methane
              - Power
## Advantages/disadvantages of high lignin content depending of transformation process

<table>
<thead>
<tr>
<th>Route</th>
<th>Transformation process</th>
<th>Value</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical</td>
<td>Enzymatic hydrolysis and fermentation for ethanol production</td>
<td>- -</td>
<td>Several</td>
</tr>
<tr>
<td>Methanization</td>
<td></td>
<td>- -</td>
<td>Several</td>
</tr>
<tr>
<td>Thermochemical</td>
<td>Fast pyrolysis</td>
<td>+</td>
<td>Brown, 2010</td>
</tr>
<tr>
<td>Gazification</td>
<td></td>
<td>+</td>
<td>Folkedahl, 2010</td>
</tr>
<tr>
<td>Liquefaction</td>
<td></td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Combustion/cogeneration</td>
<td></td>
<td>++</td>
<td>Several</td>
</tr>
</tbody>
</table>
Methanization: a local route for biomass transformation

- A local route through a silage stage giving flexibility to choose for dairy cows feeding or heat/electricity production at farm level.

- About 0.6 millions ha of biomass crops (maize, sunflower, sorghum...) planted in Germany for this purpose, just starting in France and some other EU countries...

- Need for high biomass productivity/ha with >28-30% of dm and high fibre digestibility (according to private seed sector)
Biomass valorization as biomaterials

✓ Biomaterials for building: blocks, *light weight concrete*, …

➢ Biopolymers: bioplastic composites for car industry and other novel applications…

➢ Based on previous studies on Miscanthus need for high cellulose content and long fibres

➢ To be better assessed in a new project Biomass crops for the future
Cropping systems for biomass sorghum

Debates still exist about:

- Dedicated summer crop (early spring planting) versus double-cropping systems (late planting of sorghum in June after a winter crop)?
- With or without irrigation?
- Marginal or better soils (eg South of France)?
Biomass sorghum (as summer dedicated crop)

= a sorghum producing high ligno-cellulosic biomass with a biochemical composition required by the chain value

- 20-40 t/ha dry matter result of a cycle duration of 4 to 5 months and plant height >3.5 m
- Early growth vigor and tolerance to cool temperatures for extending the cycle duration
- Lodging resistance
- Dry matter content at harvest time > 28-30%
- High WUE and NUE
- No objective for grain production

Lignin and cellulose content /fibre digestibility in phase with the requirements of transformation process
Research projects on biomass sorghum in Cirad

- ANR GrassBiofuel 2008-2011: characterization of the sorghum variability for cell wall composition (France + Mali)

- FP7 UE Sweetfuel WP1 2009-2013: breeding for biomass sorghum for temperate areas (Europe)

- Biomass crops For the Future (BFF) 2013-2020: integrated breeding project with industrial users for multi-purpose biomass sorghums in France
Two breeding programs assisted by molecular markers:
- MARS programs on B and R pools
- BCNAM on B pool
Sorghum breeding for biomass: Components of biomass accumulation

Dry biomass yield is a combination of:

- stems per m²
- internodes per stem
- internode length
- internode diameter
- water content at harvest
- specific weight
- plants/m²
- tillering
- duration of vegetative phase & phyllochron

Planting density
Genetic control & varietal aptitudes
Dwarf genes

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Breeding for biomass sorghum: optimizing the biomass production by

- Increasing the number of stem internodes
- Increasing length and diameter of stem internodes
- Increasing tillering?

⇒ Hybrids cultivars: need to optimize heterosis by the identification of heterotic pools
Increasing the number of internodes by increasing the duration of vegetative phase

- Increase BVP duration for photoperiod-insensitive sorghums (low variability)

- Use photoperiod-sensitive sorghums: broadening the working genetic base for sorghum improvement in temperate areas

- Improve tolerance to cold temperatures at seedling stage for allowing early plantings (variability exists!)
Exploring the variability of phyllochron and its effects on biomass yield

Preliminary study on 11 long-cycle photoperiodic sorghums in South of France 2010

- Phyllo 1 promotes whole plant productivity and phyllo 2 main stem productivity but they are not negatively linked ($r^2 \ 0.13$)
  => “Trade off” to find between both phyllochrons?
- To be more explored: impact of phase 1 & phase 2 duration with broader diversity
  => timing of breakpoint & internode elongation onset?
Increase the size of internodes: length and diameter

- Internode length controled by 4 major dwarf genes increasing length when dominant: DW1, DW2, DW3, DW4

- Stem diameter: huge phenotypic variability to better explore and use ex sorghum of durra race: thick stems with anchoring roots
Increasing tillering?

- A limited potential in cultivated sorghums with some genetic differences

- Start at 4-5 leaves on the main stem, stop when LAI becomes constant (about 50 dae), many tillers die during the growth

- A trait with some disadvantages in intensive agriculture thus eliminated in the grain sorghum breeding programs
Towards the definition of plant idiotype for biomass production in South of France—Evaluation of various plant architecture and phenology

- Higher biomass yield for **late but flowering** sorghums (final August/first days of September)
- Some **moderate tillering** seems to be profitable for yield
- Higher fiber digestibility for no flowering sorghum

<table>
<thead>
<tr>
<th>Accession</th>
<th>date to flag leaf</th>
<th>number of tillers at mid-cycle</th>
<th>number of tillers at harvest</th>
<th>plant height (cm)</th>
<th>DM (%)</th>
<th>Average dry weight of one plant (g)</th>
<th>ADL/NDF</th>
<th>INNDFD</th>
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</thead>
<tbody>
<tr>
<td>IS 15148</td>
<td>16-aout</td>
<td>0.07</td>
<td>0.07</td>
<td>247</td>
<td>34.3</td>
<td>196</td>
<td>7.8</td>
<td>20.0</td>
</tr>
<tr>
<td>IS 26833</td>
<td>30-aout</td>
<td>0.64</td>
<td>0.33</td>
<td>340</td>
<td>32.4</td>
<td>289</td>
<td>9.0</td>
<td>15.8</td>
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<tr>
<td>IS 4285</td>
<td>03-sept</td>
<td>0.27</td>
<td>0.27</td>
<td>290</td>
<td>25.0</td>
<td>236</td>
<td>7.4</td>
<td>22.0</td>
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<td>IS 32569</td>
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<td>1.47</td>
<td>1.40</td>
<td>308</td>
<td>29.6</td>
<td>368</td>
<td>7.7</td>
<td>20.6</td>
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<tr>
<td>IS 23777</td>
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<td>3.21</td>
<td>2.80</td>
<td>238</td>
<td>20.3</td>
<td>310</td>
<td>6.7</td>
<td>23.6</td>
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<td>IS 31559</td>
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<td>0.33</td>
<td>207</td>
<td>16.6</td>
<td>241</td>
<td>5.8</td>
<td>28.9</td>
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<td>0.00</td>
<td>0.00</td>
<td>261</td>
<td>15.7</td>
<td>155</td>
<td>6.9</td>
<td>26.7</td>
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<tr>
<td>IS 2156</td>
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<td>3.13</td>
<td>2.36</td>
<td>221</td>
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<td>226</td>
<td>7.0</td>
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<td>1.57</td>
<td>213</td>
<td>17.7</td>
<td>287</td>
<td>7.1</td>
<td>22.7</td>
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<td>IS 11026</td>
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<td>2.14</td>
<td>0.76</td>
<td>247</td>
<td>19.7</td>
<td>384</td>
<td>8.0</td>
<td>19.4</td>
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<tr>
<td>BIOMASS 140 (CH)</td>
<td>24-aout</td>
<td>1.27</td>
<td>0.40</td>
<td>317</td>
<td>30.3</td>
<td>365</td>
<td>8.2</td>
<td>18.8</td>
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<tr>
<td>total mean</td>
<td></td>
<td>1.45</td>
<td>0.94</td>
<td>263</td>
<td>23.8</td>
<td>278</td>
<td>7.4</td>
<td>21.8</td>
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<tr>
<td>Genotype effect</td>
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<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
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<td>***</td>
</tr>
<tr>
<td>flowering</td>
<td></td>
<td>1.13</td>
<td>0.97</td>
<td>285</td>
<td>28.3</td>
<td>280</td>
<td>7.7</td>
<td>20.4</td>
</tr>
<tr>
<td>not flowering</td>
<td></td>
<td>1.79</td>
<td>1.01</td>
<td>230</td>
<td>17.9</td>
<td>258</td>
<td>7.0</td>
<td>23.8</td>
</tr>
</tbody>
</table>

**ADL/NDF** and **INNDFD** indicate digestibility measurements.
Biomass quality concerns

- Cultivars with high lignin content are useful for thermochemical routes but not for biochemical (enzymatic) routes.

- For methanization and animal feeding, need to develop biomass cultivar with high degradability of cell wall:
  - low lignin content: use of bmr genes
  - understand better the effect of the different lignin (S and G) and p-coumaric and ferulic acids, and the influence of the localization of lignins in the tissues on cell wall degradability.

Lodging concern: breeding for very low lignin content seems to increase lodging problems in certain conditions.
The Grass Cell Wall: Complex Hetero-Polymers

**Phenolics**
- p-Hydroxycinnamic acids
  - p-Hydroxyphenyl (H) 4%
  - Guaiacyl (G) 45%
  - Syringyl (S) 61%

**Monolignols**
- Ferulic acid
- p-Coumaric acid
- H bounds

**Carbohydrates**
- Cellulose
- ArabinoXylans

**With Complex Cross-linkages**
- ArabinoXylans
- Cellulose
- Ferulic acid
- pCoumaric acid
A variability in stem composition to be better characterized

Evaluation of 100 accessions from the Cirad Core Collection flowering in South of France

<table>
<thead>
<tr>
<th>Stem composition parameters</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDF (% dm)</td>
<td>35-82</td>
</tr>
<tr>
<td>ADF (% dm)</td>
<td>19-51</td>
</tr>
<tr>
<td>ADL (% dm)</td>
<td>1.2-8.7</td>
</tr>
<tr>
<td>ADL/NDF ratio</td>
<td>3-11</td>
</tr>
</tbody>
</table>
**A racial structure for variability in stem composition?**

<table>
<thead>
<tr>
<th>Genetic Cluster*</th>
<th>Number</th>
<th>DM</th>
<th>NDF</th>
<th>ADL</th>
<th>ADL/NDF</th>
<th>CELNDF</th>
<th>INNDFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Durra Africa &amp; Asia</td>
<td>16</td>
<td>33.35</td>
<td>58.32</td>
<td>4.64</td>
<td>7.91</td>
<td>51.24</td>
<td>17.73</td>
</tr>
<tr>
<td>Caud et Bicolor China</td>
<td>11</td>
<td>34.06</td>
<td>73.86!</td>
<td>6.81!</td>
<td>9.19</td>
<td>51.02</td>
<td>12.32</td>
</tr>
<tr>
<td>Caud Africa no photop</td>
<td>22</td>
<td>26.79</td>
<td>63.09</td>
<td>4.75</td>
<td>7.53</td>
<td>51.03</td>
<td>19.28</td>
</tr>
<tr>
<td>Kafir Southern Africa</td>
<td>29</td>
<td>28.20</td>
<td>61.44</td>
<td>4.95</td>
<td>8.04</td>
<td>51.50</td>
<td>18.36</td>
</tr>
<tr>
<td>Guinea Southern Africa</td>
<td>6</td>
<td>39.65</td>
<td>61.69</td>
<td>5.37</td>
<td>8.69</td>
<td>51.19</td>
<td>14.01</td>
</tr>
<tr>
<td>Bicolor world (except China)</td>
<td>9</td>
<td>29.50</td>
<td>63.62</td>
<td>5.20</td>
<td>8.17</td>
<td>51.36</td>
<td>16.51</td>
</tr>
<tr>
<td>Cluster effect</td>
<td></td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
</tr>
</tbody>
</table>

* Genetic cluster according to Deu et al. 2006

! significant different with other cluster at \( p < 0.05 \) (lsd test)

- **Significant effect of genetic cluster for all parameters except cellulose content**
- **Chinese sorghums sources of high total fiber (NDF) and high lignin**
- **No photoperiodic Caudatum from Africa and Kafir source of high stem digestibility**
No genetic limitations to improve both yield and digestibility

- It seems possible to develop high yielding biomass sorghums with high fibre digestibility
In-depth biochemical analysis of the cell wall composition of 13 genotypes representative of the sorghum biochemical stem variability (NIRS profile)

Variability of cell wall components for a sub-panel representative of the worldwide sorghum diversity

<table>
<thead>
<tr>
<th>Trait</th>
<th>mean</th>
<th>Cv %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall residue (% DM)</td>
<td>70.32</td>
<td>14.83</td>
</tr>
<tr>
<td>Lignin Klason content (% CWR)</td>
<td>16.32</td>
<td>9.05</td>
</tr>
<tr>
<td>G μmol/g LK</td>
<td>407.00</td>
<td>10.57</td>
</tr>
<tr>
<td>S μmol/g LK</td>
<td>361.01</td>
<td>19.29</td>
</tr>
<tr>
<td>Lignin S/G</td>
<td>0.89</td>
<td>14.71</td>
</tr>
<tr>
<td>Esterified p-coumaric acid (mg/g CWR)</td>
<td>16.13</td>
<td>12.18</td>
</tr>
<tr>
<td>Esterified ferulic acid (mg/g CWR)</td>
<td>4.75</td>
<td>8.89</td>
</tr>
<tr>
<td>Etherified ferulic acid (mg/g CWR)</td>
<td>4.06</td>
<td>9.60</td>
</tr>
<tr>
<td>Total Sugars (% CWR)</td>
<td>75.56</td>
<td>4.35</td>
</tr>
<tr>
<td>Arabinose (% CWR)</td>
<td>4.58</td>
<td>21.21</td>
</tr>
<tr>
<td>Galactose (% CWR)</td>
<td>1.09</td>
<td>40.43</td>
</tr>
<tr>
<td>Glucose (% CWR)</td>
<td>41.18</td>
<td>14.81</td>
</tr>
<tr>
<td>Xylose (% CWR)</td>
<td>28.72</td>
<td>10.74</td>
</tr>
</tbody>
</table>

Selection of the 13 accessions among 384 to represent the sorghum stem biochemical variability

➢ A significant variability for all the cell wall components was detected

Source: D. Pot et al., 2011. ANR Grassbiofuel project
Effects of Alkaline Pretreatment

- Alkaline pretreatments improved significantly the conversion yields of cellulose and arabinoxylan.
- but they did not eliminate the genetic variability.

Source: D. Pot et al., 2011. ANR Grassbiofuel project
Breeding for biomass sorghum is still recent but may take advantage of the great variability existing in sorghum and novel genetic tools.

One quiet well defined target: biogas-silage.

For 2G-ethanol processes, several locks to be broken:

- Biomass composition (cell wall) need to be optimized.
- Technological challenges: set up viable industrial processes (low-cost pretreatments, enzymatic cocktails, ...).
- Economic rentability of the chain need to be proved.

Several other opportunities for utilization of biomass sorghums as biomaterials and bioplastics...
THANK YOU FOR YOUR ATTENTION!