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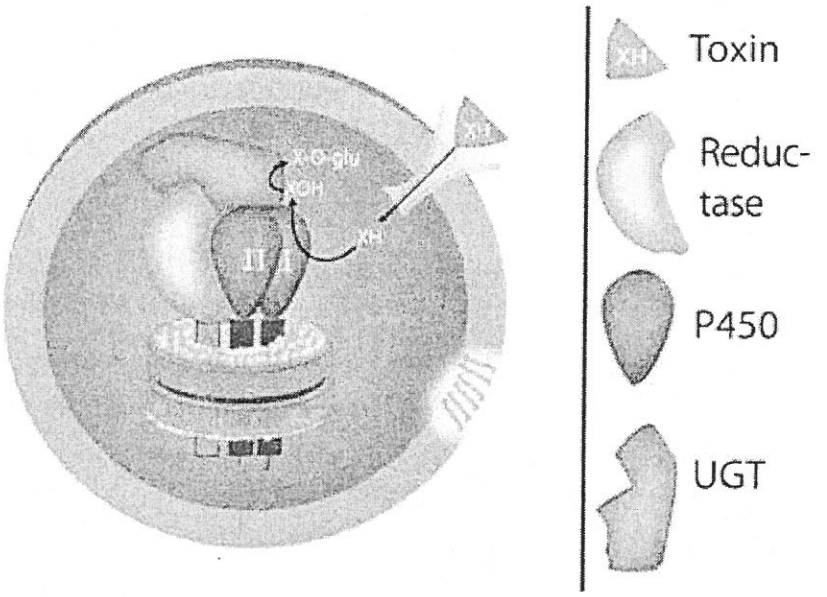

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Fructans in Brachypodium distachyon as an alternative carbon sink to lignin

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We present a newly started project, where the concept is to change carbon flux away from lignin and in direction of production of soluble degradable storage polysaccharides. This will be done by genetic transformation of *Brachypodium distachyon* 1) to gently reduce lignin biosynthesis by targeting DAHP synthase and 2) to introduce genes encoding fructosyl transferases, that will synthesize fructans as a carbon storage in leaves.

Along with this we will select and express genes encoding fructan hydrolases in microorganisms, in order to obtain new enzymes suitable for degrading the fructans stored in grass leaves.

Fructans in *Brachypodium distachyon* as an alternative carbon sink to lignin

DET JORDBRUGSVIDENSKABELIGE FAKULTET AARHUS UNIVERSITET

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The concept is to ...

- change carbon flux in direction of production of soluble degradable storage polysaccharides by introducing fructosyl transferases and gently reducing lignin biosynthesis.
- express new fructanases to select enzymes suitable degrade the stored fructans

The project is a part of a new initiative "Bio4Bio".

Bio4Bio

"A strategic centre for the development and implementation of biotechnology for bioenergy " funded by **Danish Council for Strategic Research**

Focus:
lignocellulosic plant biomass as a resource for making renewable liquid fuels.

The centre will coordinate research groups working within plant biology, biomass technology and microbiology for optimal conversion of non-food plant biomass. The research objectives are organized in the following Work Packages:

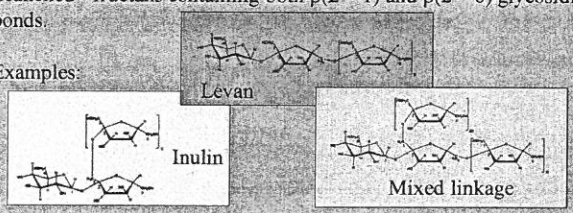
Biomass selection and development
Exploiting potential in existing material
Core technologies - including:
Plant transformation (Brachypodium distachyon),
Saccharification screening
Characterization of plant and cell wall structural features
Protein Discovery - including:
New Cell wall degrading enzymes and accessory proteins,
New fructanases
Functional genomics - Including:
Targeted approaches using plant transformation
Production and selections of TILLING mutants
Testing and up-scaling

Fructans

Polysaccharides composed of fructose units linked by β(2→1) and β(2→6) glycosidic bonds. In many plants fructans represent a major carbon storage.

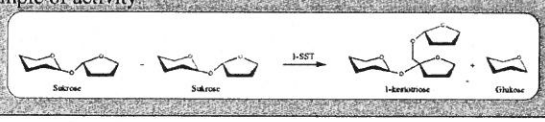
Fructans occur as different types:
Inulin - linear fructans generally linked by β(2→1) glycosidic bonds
Levan - linear fructans generally linked by β(2→6) glycosidic bonds
Branched - fructans containing both β(2→1) and β(2→6) glycosidic bonds.

Examples:



Fructans are synthesized by fructosyl transferases. The transfer of a fructose from either sucrose (SST, SFT) or in between fructan chains (FFT). A range of transferases with different specificities occur and give rise to fructans with different structure and DP.

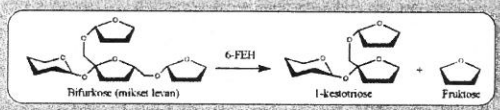
Example of activity:



Fructanases

Fructans are degraded by hydrolysis by specific fructan exohydrolases (FEH)

Inulase (1-FEH) will cleave β(2→1) glycosidic bonds and whereas 6-FEH cleaves β(2→6) in levans.



Inulase is commercially available whereas 6-FEH is not

Task:
- Isolate genes encoding fructosyl transferases from Brachypodium and from other plant sources.

- Make constructs for overexpression of fructosyl transferases in Brachypodium.

- Analyze the natural fructan metabolism in Brachypodium.

Task:
1) Isolate genes encoding 6-FEH and express these in microorganisms.

Goal:
To be used for fructan degradation and as analytical tool.

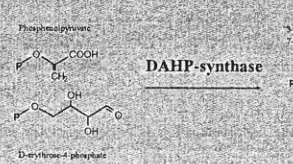
Brachypodium distachyon.

Brachypodium will be used as a model plant for temperate grasses. Transformation techniques will be established to modify plants.
Temperate grasses can store large amounts of fructans in leaves, typically during cold stress and other conditions leading to sugar accumulation in the leaves. Brachypodium is not yet characterized in this respect.

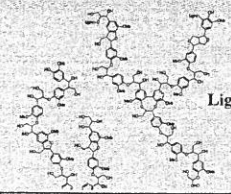
Task:
Reliable procedures for transformation of Brachpodium are now established

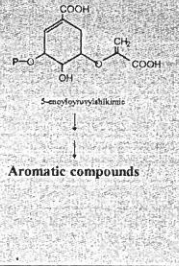
Task & Goal :
Transformation of Brachypodium to obtain over-accumulation of fructans and reduced lignification in leaves.

Lignin



The entry to synthesis of lignin is catalyzed by DHAP synthase (the target enzyme of glyphosate).





Task:
Identify the genes encoding DHAP synthase in Brachypodium, and make constructs for reduced expression in Brachypodim.

Pro-Active Plants