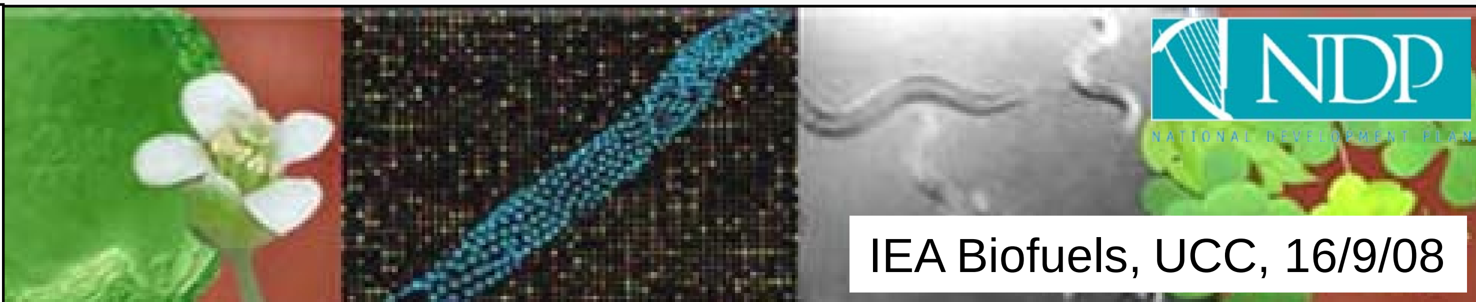
Genetics & Biotechnology for Sustainable Biofuel Systems

Dr. Charles Spillane
Genetics & Biotechnology Lab
Dept of Biochemistry & Biosciences Institute
University College Cork
Ireland.
c.spillane@ucc.ie



IEA Biofuels, UCC, 16/9/08

The interlinked mega-challenges

+

Population +1.2% p.a.

Food +100% by 2050

+ -

Energy +1.8% p.a.

Climate +2^{o} by 2050

-

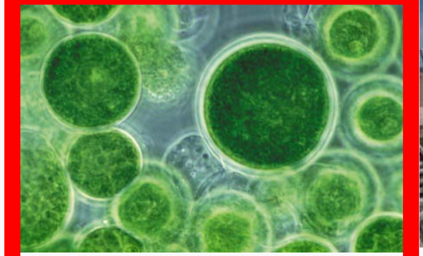
Source: Patrick Cunningham, Chief Scientist (Ireland)

Renewable energy portfolio

WAVE

WIND

TIDAL



BIOMASS

HYDRO

Current global energy demand: 13 TW

Global energy demand by 2025 = 22 TW

(11 TW of which needs to come from clean energy if we are to respect the 450 ppm $CO2$ emissions limit)

Not much time!!

=> Pursue all options in portfolio….

PV

SOLAR TOWER

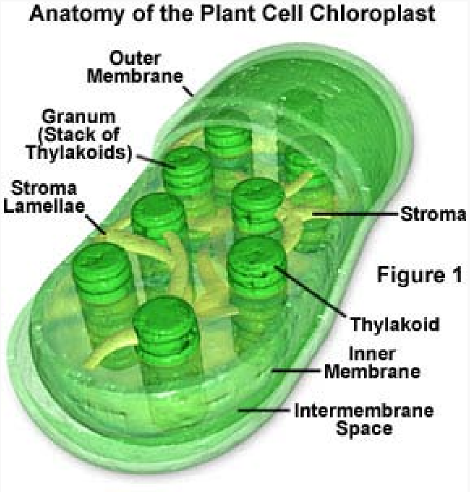
Source: Chris Bowler

Planetary challenges facing humanity

(1) Food & feed production/access

(2) Fossil fuels transition → renewable energy

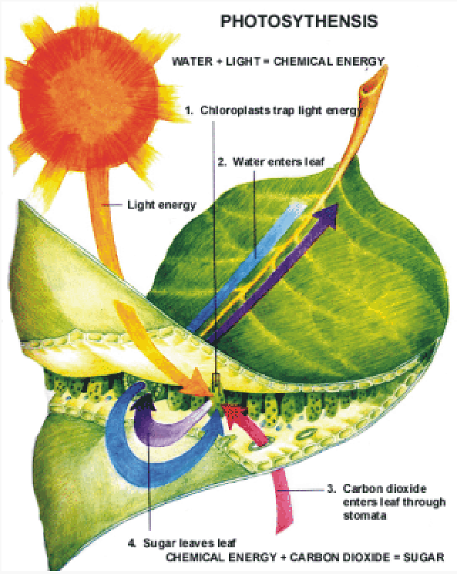
(3) CO_2 recycling & sequestration



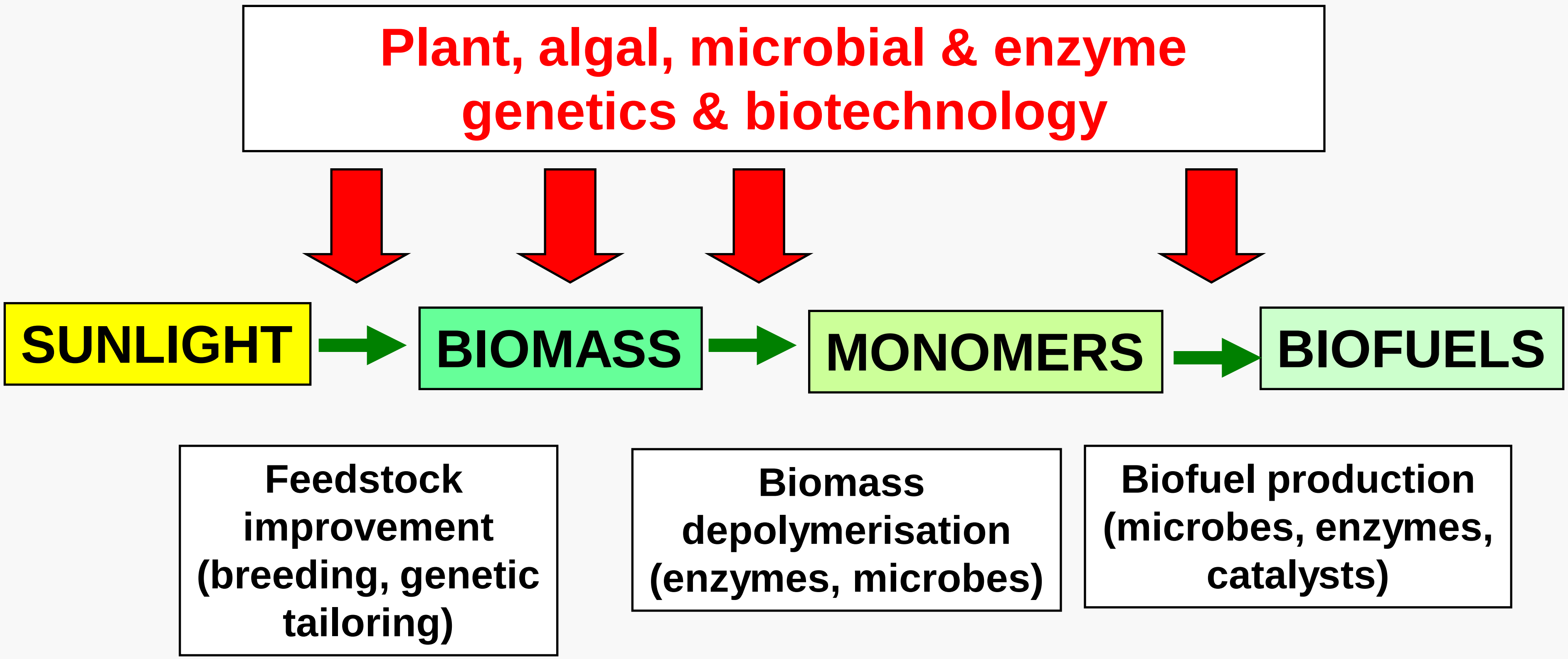
Photosynthesis is critical to meeting these challenges

$$CO_2 + 2H_2O + 8 \text{ photons}$$

$$CH_20 + H_20 + O_2$$



Genetics & biotechnology for sustainable bioenergy



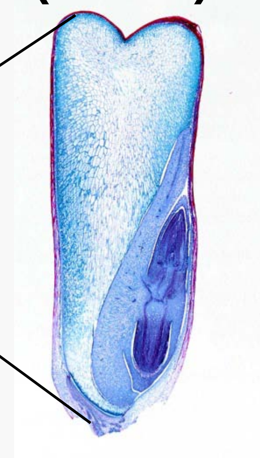
Integrated approach:

⇒ focus on “biological” nodes of process chain that can be genetically improved and tailored towards bioenergy & co-products.

=> Large inter-disciplinary research programmes necessary.

Crop domestication for food, feed & fibre

Corn Grain (starch)



tomato ← Lycopersicon

No crop, algal species, microbe or enzyme has been fully "optimised" for bioenergy production

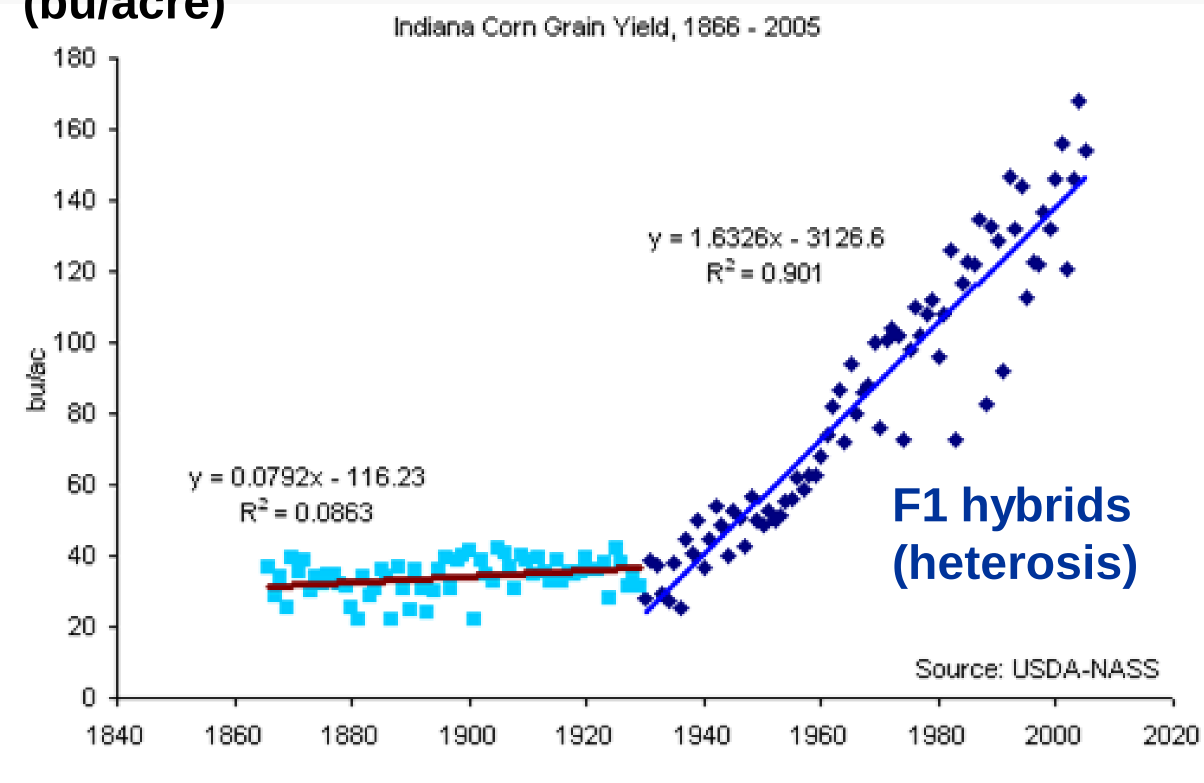
Need for accelerated "re-domestication" of plants, algae, bacteria & enzymes for bioenergy production.

Can we reduce 10,000 years → a few decades???

Genetics: Plant breeding to develop higher yielding crop varieties

Hybrid genetics has tripled US maize yield since 1940

yield (bu/acre)

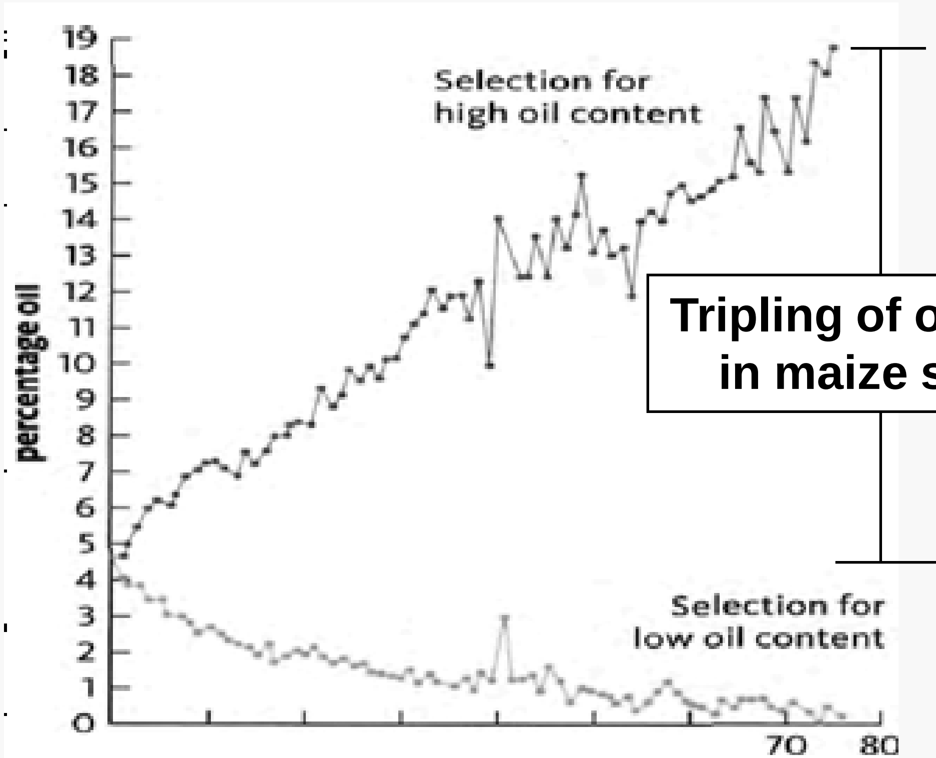


1940 ⟶ today

Heterosis (hybrid vigor)

B73 F1 hybrid Mo17

Genetics: Plant breeding to develop genetically tailored crop varieties



Tripling of oil yield in maize seeds

1896

~ 90 generations of maize breeding

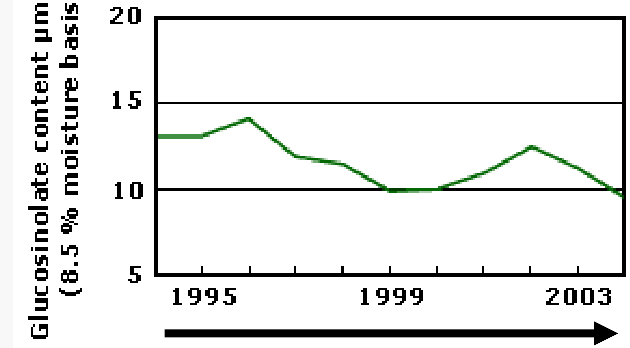
Oilseed rape (Canada)

<u>Can</u>adian, <u>l</u>ow <u>a</u>cid oil

Canola

↓

Low glucosinolates
Low erucic acid



New varieties with reduced toxicity

Conventional plant breeding to market (farmer, consumer) timescales are long.

Breeding → Advanced trials → Seed multiplication/certification → Crops in ground

Genetic tailoring of photosynthetic organisms for bioenergy is in its infancy

None of today's energy crops, organisms or enzymes have had significant breeding/genetic improvement for specific bioenergy applications.

Crops which have been subjected to significant breeding efforts have been mainly for food and feed applications (i.e. not optimised for bioenergy).

Miscanthus x giganteus (3x)	**Maize**	**Soybean**
Reed canary grass (*Phalaris*)	**Sugarcane**	**Jatropha**
Switchgrass (*Panicum*)	**Sweet sorghum**	**Oilseed rape**
	Energycane	**Oil palm**

Poplar (*Populus*)
Willow (*Salix*)
Eucalyptus
Bamboo
Pines

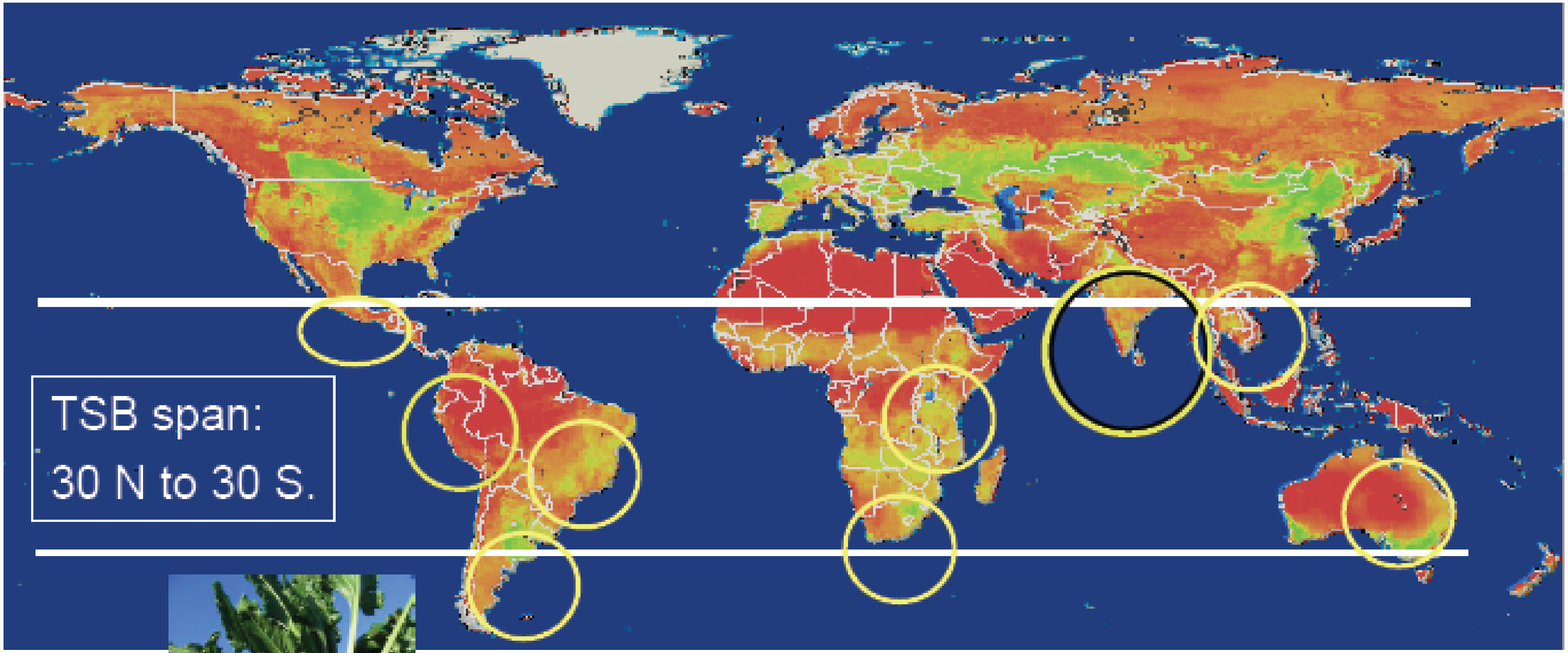
What about new plants/organisms for bioenergy?

Microalgae? Macroalgae? Cacti? Moringa…

Cyanobacteria? (*Synechocystis*….)

Many new & underutilised species……..

Tropical sugar beet – a new bioenergy crop



Strengths
- Water – 33% to -50% vs sugarcane
- Fit to saline/alkaline soils
- Cost competitive with sugarcane
- Increased farmer income
- Provides an additional cash crop

Development time ~ 10 years

syngenta

Extensive genomics & gene discovery underway for bioenergy relevant organisms (Rubin, 2008)

Genome sequencing & genomics programs underway for most bioenergy crops

JGI DOE JOINT GENOME INSTITUTE US DEPARTMENT OF ENERGY OFFICE OF SCIENCE

DOE Joint Genome Institute
Enabling Bioenergy Advances

Microalgal genome sequenced (*Chlamydomonas*)

Microbial biomass degraders – at least 11 genomes sequenced & many more in progress

Metagenomics of termite hindgut & other cellulolytic environments

Microbial fuel producers – at least 6 genomes sequenced & many more in progress

Gene discovery → Functional analysis of gene-trait associations

Genetic traits for improving energy crops

Stand Establishment
- Germination rate
- Cold germination
- Cold growth
- Herbicide tolerance

Nitrogen Economy
- Uptake and translocation
- Reduction and partitioning

Stress Tolerance
- Drought tolerance and recovery
- Heat
- Cold
- Salt
- Photoperiod

Improved Processing
- Lignin content
- C6/C5 ratio
- Cell structure
- Organ structure

Hormone biology
- Brassinolides
- Gibberellins
- Auxin

Photosynthesis
- Planting density
- Light harvesting / utilization
- Chloroplast position and number
- PEP carboxylase, SBPase, FBPase

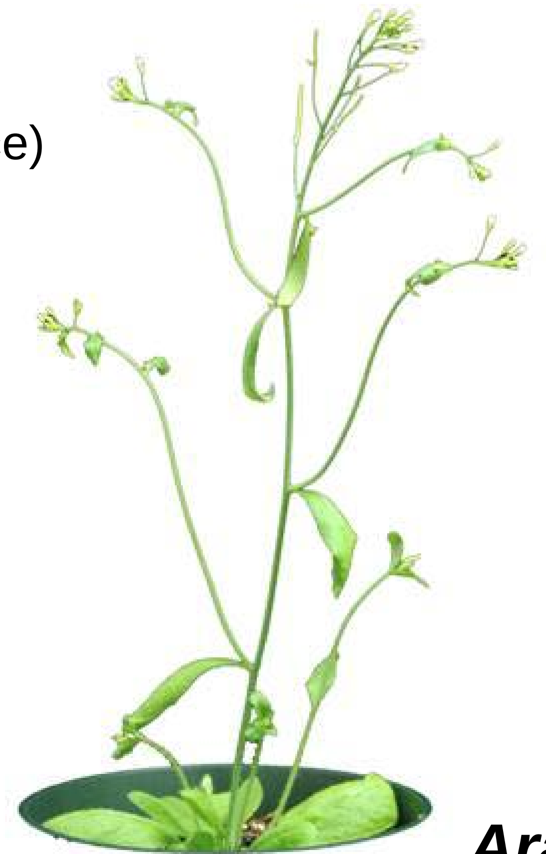
Plant Architecture
- Branching
- Stalk thickness
- Root length
- Stature
- Leaf size / angle

Reproduction
- Heterosis
- Genetic confinement

Richard Flavell

Use model organisms to functionally screen for high-priority traits

- Drought (including surrogates)
- Low Nitrogen (including surrogates)
- Cold and Freezing
- Heat (all stages)
- Light (e.g., shade tolerance)
- UV tolerance
- Photosynthetic efficiency
- Low pH and aluminum
- High pH
- Growth rate
- Flowering time
- Stay green and maturity
- Plant architecture
- Fertility
- Organ size
- Stature
- Stalk thickness



- Ozone
- High CO_2
- High Nitrogen
- Carbon/Nitrogen
- Seed morphology
- Biotic, fungal
- Composition
  - seed oil
  - seed protein
  - lignin
  - sterols
- and others

Arabidopsis thaliana

Richard Flavell

gene-trait associations in model plants

Drought tolerance

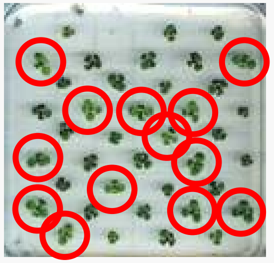
Increased yield

Heat tolerance

Drought recovery

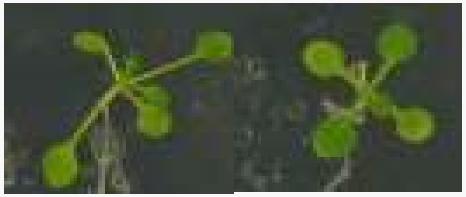
Nutrient utilization

Root growth



Cold germination

Increased biomass



Shade tolerance

Flowering time

Stature control

Salt tolerance

Genetics & disruptive innovations in bioenergy sector?

The food-energy-population-climate challenge urgently needs revolutionary innovations from S&T.

Genetics & biotechnology will have a role in delivering:

- **Ethanol & other biofuels from cellulosics**
- **Bioenergy-tailored enzymes**
- **Designer bioenergy crops**
- **New forms of oil and lipid supply from biomass**
- **Bacteria & algae domesticated for bioenergy applications**
- **New forms of liquid biofuels with higher energy content**
- **Bio-refineries for co-production of energy, chemicals, etc**

Genetics & biotechnology role in transition to a renewables bio-based economy (glucose economy, hydrogen economy)

2º - Liquid biofuels from cellulosic biomass

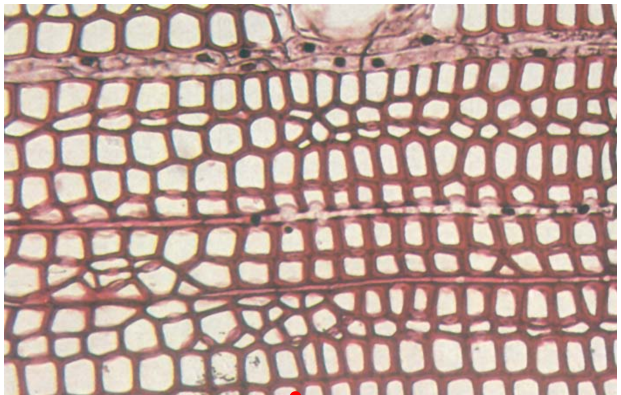
High-cost of processing!

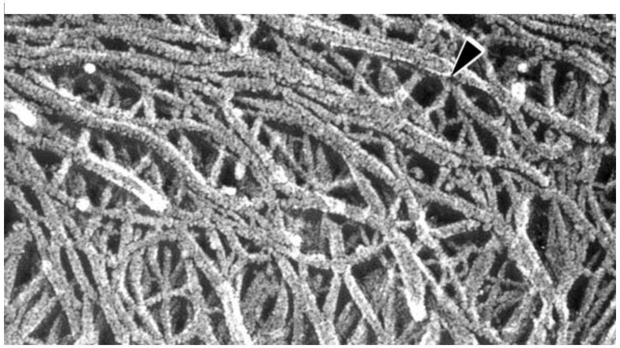
Cellulose

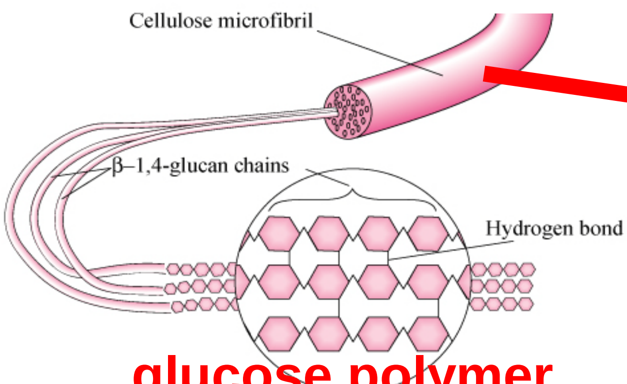
linear D-glucose polysaccharide

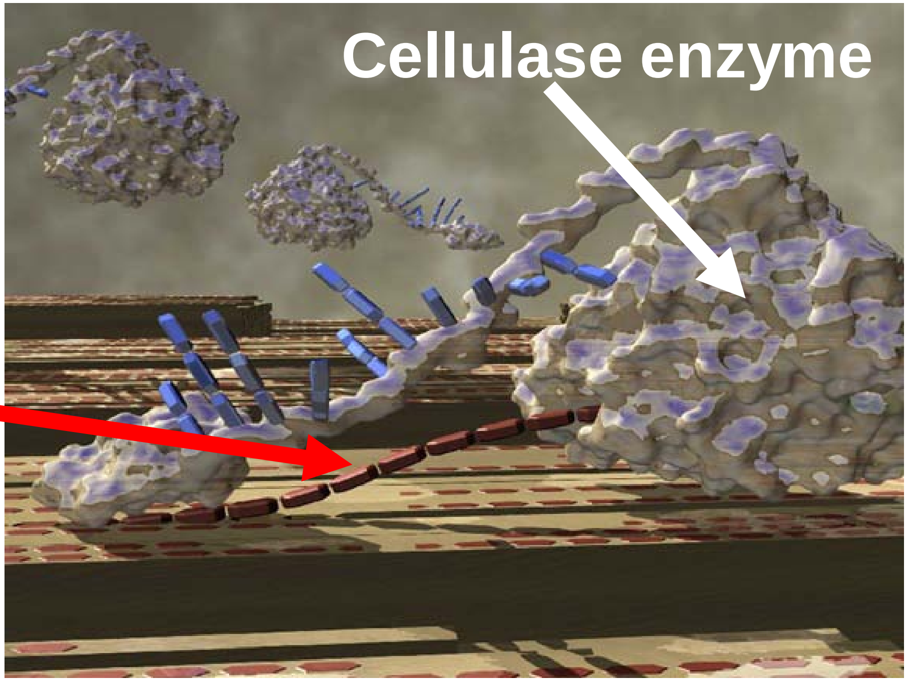
~40% of plant biomass, cell walls

recalcitrant to hydrolysis



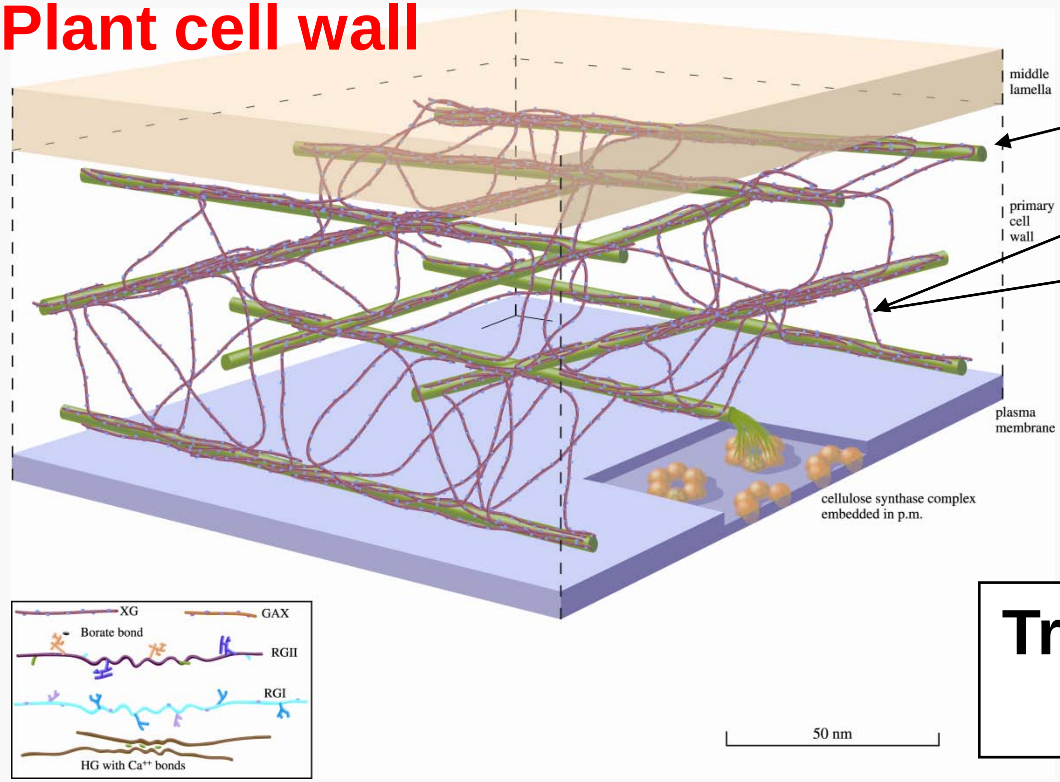






Hemi-cellulose & lignin occlude access to cellulose

Plant cell wall



Cell wall composition

Cellulose (40-50%)
Hemicellulose (20-30%)
Lignin (15-25%)
Pectins (10-20%)
Proteins (<10%)

Transgenics, mutagenesis, natural variation

2 complementary approaches:

1) Genetics to modify plants to increase processability

+

2) Genetics to design/develop better enzymes (cellulases, etc)

Genetics to develop improved cell wall degrading enzymes

- Biomass degrading fungus (*Trichoderma reesei*) – major source of **biomass degrading enzyme cocktails**

- Genome sequenced (2008), mutant strains next (DoE JGI)

- Combinatorial cocktails of > 2000 enzymes → 4 enzymes

VERENIUM
THE NATURE OF ENERGY™

<u>Targets</u>

↓ Cost

↓ Dose

↑ Activity

↑ Expression

Stability

No inhibitors

(1) Gene discovery

- New enzymes in Nature shaped by evolution?

- Where biomass degraded? Termite gut

- Metagenomics & high-throughput (millions) screens

(2) Accelerated evolution / genetic engineering

- Make & test millions of "new" gene/enzyme variants

- Identify enzymes that improve on Nature's best

3º Energy crops as self-processing feedstocks

Why make, purify & re-add enzymes to biomass....
....if bioenergy crop can make the enzymes *in planta*?

Can make any enzyme/protein in specific crop cells/tissue, developmental stages or in response to inducers (e.g. heat)

=> Transgenic crops (corn, cane, beet) whose cells produce enzymes at appropriate stage of bioenergy processing chain.
(exo-cellulases, endo-glucanases, β-glucosidase, hemicellulases)

e.g. Transgenic maize producing α-amylase in kernels

- $\uparrow$ ethanol per bushel
- $\uparrow$ plant throughput
- $\downarrow$ energy/water use
- $\downarrow$ chemical usage

syngenta

What about plant oils?

Ethanol not an ideal energy vector (energy density low)

	Energy density (MJ/L)	Energy per CO_2 (MJ/kg)
Ethanol	18.4-21.2	12.2-14.0
Methane	23-23.3	20-20.3
Butanol	29.2	15.2
Biodiesel	33.3-35.7	13.3
Hydrogen	8.5-10.1	
Petrol	32-34.8	13.6-14.6
Diesel	40.3	14.2

Oilseed crop yields?

Oil yields	Litres/ha-yr
Soybean	400
Sunflower	800
Canola	1,600
Jatropha	2,000
Palm oil	6,000
Microalgae*	40,000

* *Maximum potential for Arizona (USA)*

3º Beyond oilseeds => Oils in non-seed tissues?

Increase energy density of harvested biomass via oil production

Metabolic re-engineering of oil biosynthesis pathways in crops

Oil production in roots of transgenic rutabago (swede)

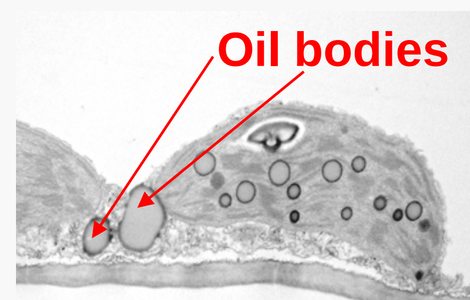
GLBRC
Great Lakes Bioenergy Research Center

Christoph Benning (MSU)

Blocking fatty acid breakdown during leaf senescence => oil accumulation in leaves

$\Rightarrow$ Get this to happen in genetically modified crop before/after harvest?

If silage from forage grasses was 10% oil?



Ian Graham
(Uni of York)

CNAP

3º Microalgae genetics & biotech for biofuel

- Microalgae species diverse (~40,000 species)
- Efficient, rapid growth, double biomass in a day
- Biomass yield 5-10x ↑ Energy yield 6-12x ↑
- Miscanthus 10t/ha-yr, microalgae 50t/ha-yr
- No cellulose or lignin
- No competition for arable land. Water?? (halophilic microalgae)
- 20 million acres => all US transportation fuel (970 million arable acres)
- Genetics & biotechnology of microalgae for biofuel in its infancy

<u>What's needed for microalgal biorefineries?</u>

- Develop improved strains (biomass, energy yields)
- Develop genetic & molecular toolbox for microalgae

=> Accelerated domestication of microalgae!

- Improved cultivation systems

- Improved fuel recovery systems

4º Synthetic biology for new bioenergy organisms & more energy-dense biofuels?

Synthetic biology – design new organisms & biosynthetic pathways for bioenergy (metabolic engineering)

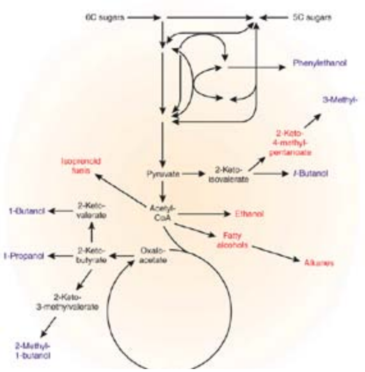
Jay Keasling

Move pathway for anti-malarial arteminisin (plants → bacteria)

AMYRIS BIOTECHNOLOGIES

Engineer GM microbes to produce more energy dense biofuels (jet-fuels)

- Butanol (traditionally *Clostridium acetobutylicum*) → *E.coli* (Inui, 2008)
- Re-engineer *E.coli* (2 enzymes) produce higher alcohols (Atsumi, 2008)
- Produce microdiesel from GM *E.coli* (Kalscheuer, 2006)
- Isopentanol production in GM *E.coli* (Withers, 2007)
- Isoprenoid derived biofuels (alkanes, alkenes, alcohols)



SYNTHETIC GENOMICS Craig Venter

George Church LS9, INC.

Conclusions

Genetics & biotechnology to harness photosynthetic organisms for sustainable bioenergy.

Integrated multi-disciplinary R & D with large budgets have highest odds of success (EBI, JBEI, Porter Alliance).

Potential for disruptive innovations => modular approach to bioenergy engineering

Green/organic movements mis-informed “gene-phobia” a barrier for sustainable bioenergy systems (e.g. GM free)

Unique opportunity for inter-discipinary research between biosciences, chemistry, engineering etc

Genetics & biotechnology at core of co-production necessary to make bio-refineries economically viable.

END

questions?

c.spillane@ucc.ie