Carbon pathways in sugarcane and miscanthus : *targets to improve them artificially*

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Feedstock biochemstry applied to Biofuels, Fulbright, 2009

The wall in the context of plant composition

Obtained from CO_2 and water

96%
- Carbon...45%
- Oxigen...45%
- Hidrogen.......................................6%

Cellulose, hemicelluloses & pectins 96-10%=86%

Macronutrients

3.6%
- Nitrogen.......................................1.5% X 6.25 = 9.4% (10%)
- Potassium......................................1.0% X
- Calcium..0.5%
- Magnesium......................................0.2%
- Phosphorous....................................0.2% X
- Sulfur...0.1% X
- Silicium.......................................0.1%

Proteins and Nucleic acids

Pectins = 0.7%

Pectins? = 0.7%

Micronutrients

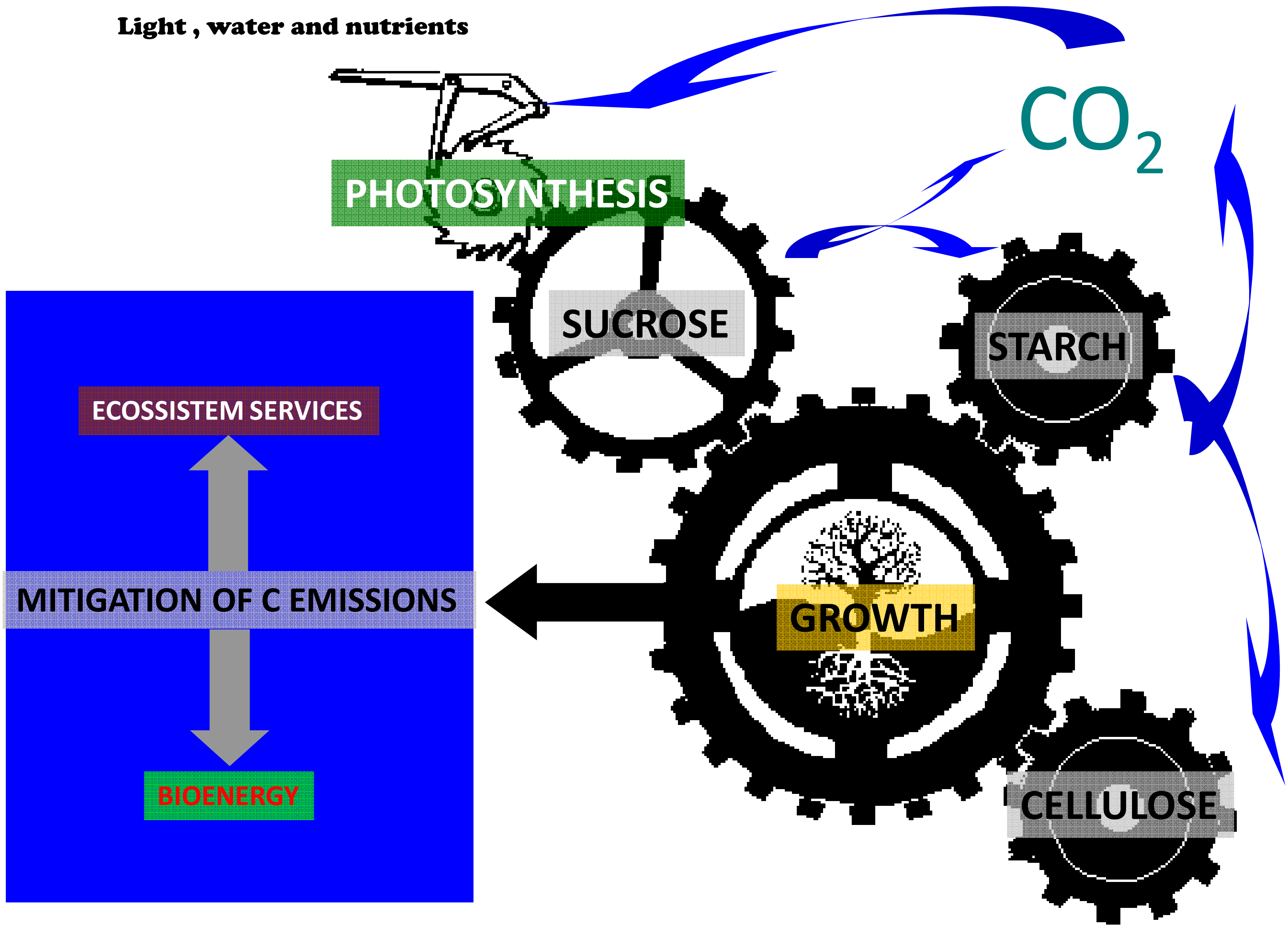
0.4%
- Boron..............................
- Manganese..........................
- Chloride........................... X
- Iron............................... X
- Sodium............................. X
- Zinc............................... X
- Copper............................. X
- Nickel............................. X
- Molibdenium........................ X

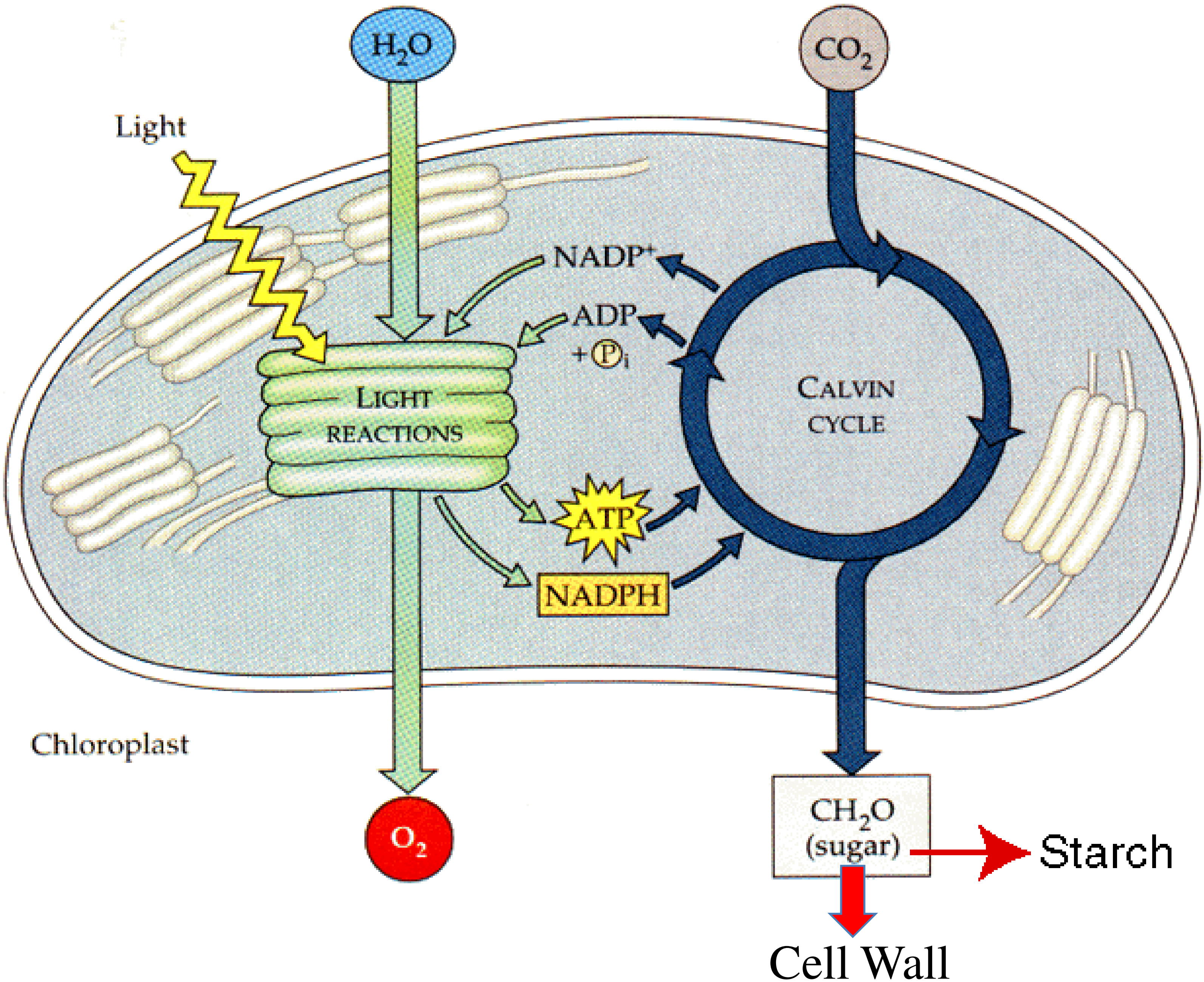
Pectins = traces

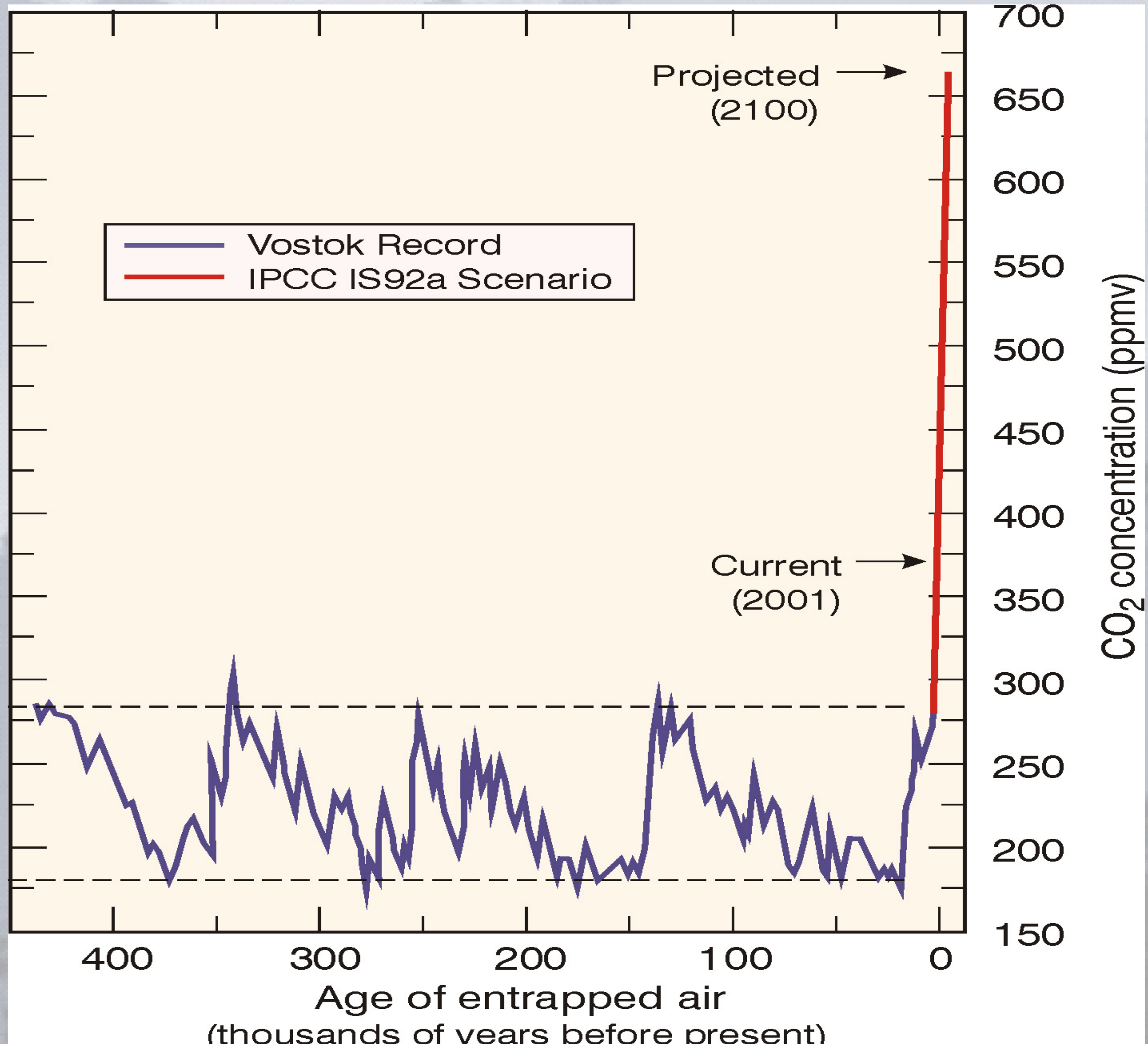
Lipids are approximately 15% of plant tissues

Thus, the wall corresponds to ca. 70 % of the plant

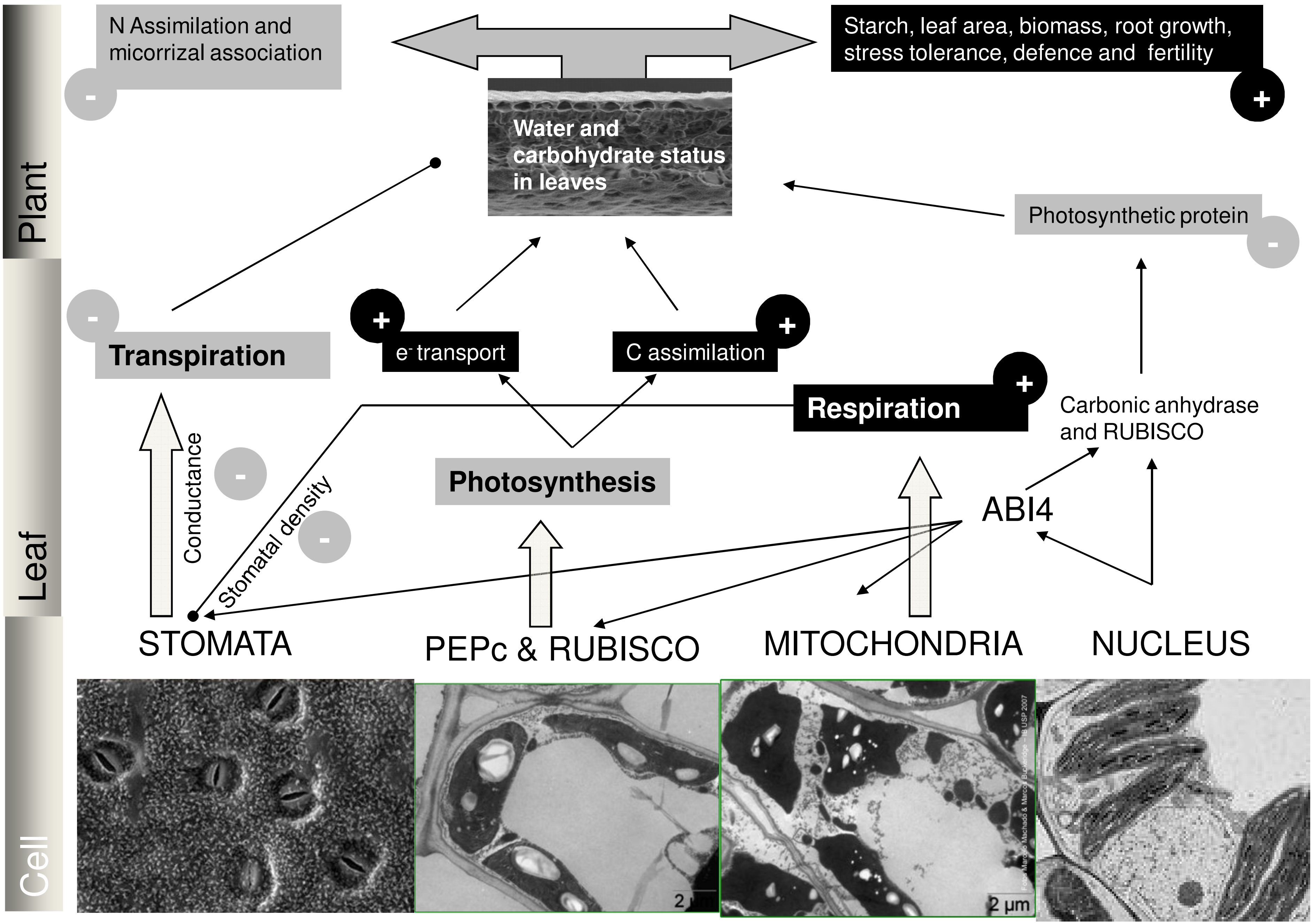
In sugarne = leaves contain 68% and stem 50% plus 18% of sucrose


Light , water and nutrients
CO_2
PHOTOSYNTHESIS
SUCROSE
STARCH
GROWTH
CELLULOSE
ECOSSISTEM SERVICES
MITIGATION OF C EMISSIONS
BIOENERGY


H2O
CO2
Light
NADP+
ADP
+ Pi
LIGHT REACTIONS
CALVIN CYCLE
ATP
NADPH
Chloroplast
O2
CH2O (sugar)
Starch
Cell Wall



Projected
(2100)
Current
(2001)
Vostok Record
IPCC IS92a Scenario
700
650
600
550
500
450
400
350
300
250
200
150
CO_2 concentration (ppmv)
400
300
200
100
0
Age of entrapped air
(thousands of years before present)



Plant
Leaf
Cell
N Assimilation and micorrizal association
-
Starch, leaf area, biomass, root growth, stress tolerance, defence and fertility
+
Water and carbohydrate status in leaves
Photosynthetic protein
-
-
Transpiration
+
e- transport
C assimilation
+
Respiration
+
Carbonic anhydrase and RUBISCO
Conductance
-
Stomatal density
-
Photosynthesis
ABI4
STOMATA
PEPc & RUBISCO
MITOCHONDRIA
NUCLEUS
2 µm
2 µm

Plant, Cell and Environment (2008) **31**, 1116–1127 doi: 10.1111/j.1365-3040.2008.01822.x

Elevated CO_2 increases photosynthesis, biomass and productivity, and modifies gene expression in sugarcane

AMANDA PEREIRA DE SOUZA[1], MARILIA GASPAR[3], EMERSON ALVES DA SILVA[3], EUGÊNIO CÉSAR ULIAN[4], ALESSANDRO JAQUIEL WACLAWOVSKY[2], MILTON YUTAKA NISHIYAMA JR.[2], RENATO VICENTINI DOS SANTOS[5], MARCELO MENOSSI TEIXEIRA[5], GLAUCIA MENDES SOUZA[2] & MARCOS SILVEIRA BUCKERIDGE[1]

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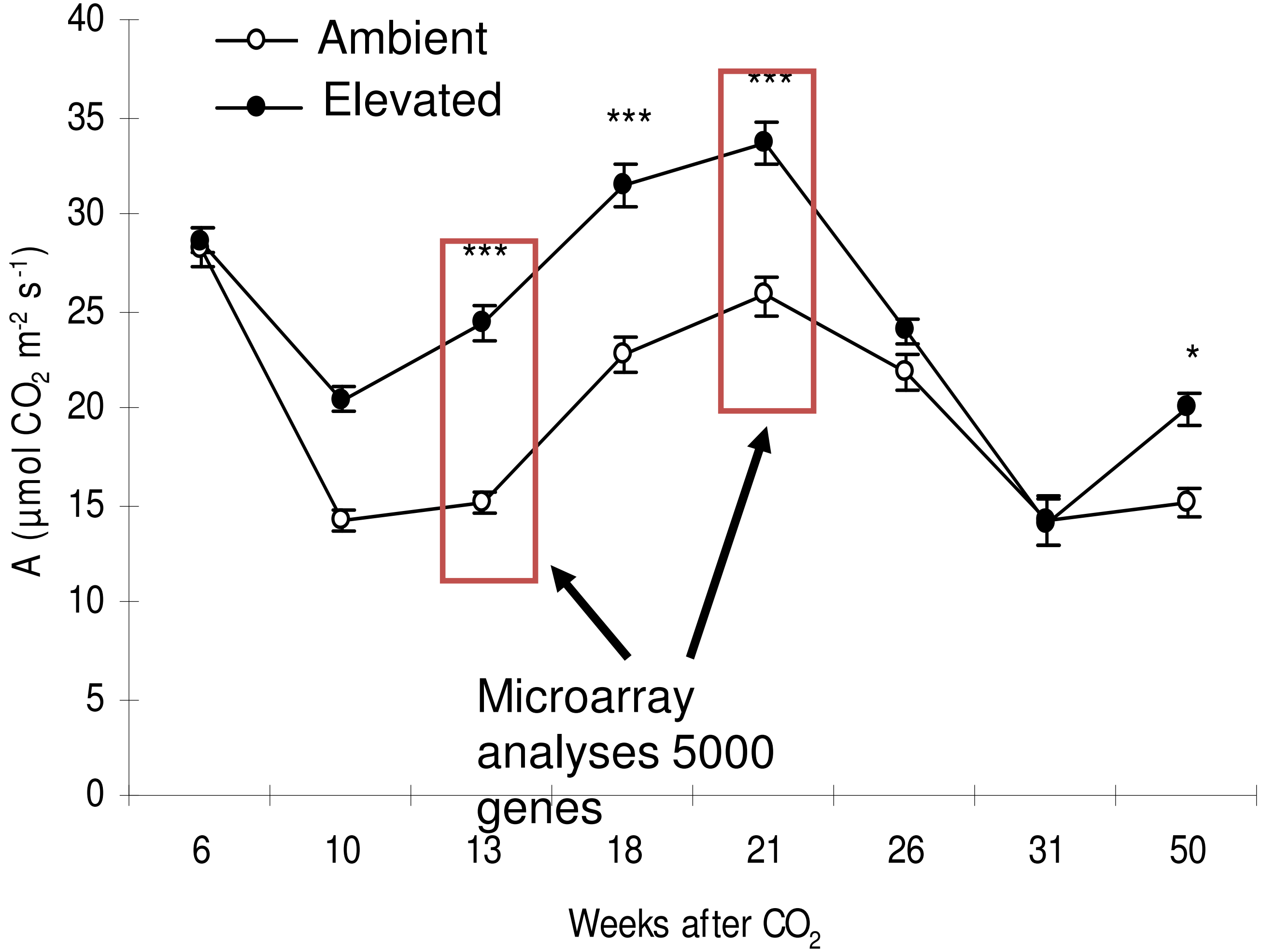
Sugar cane in the open top chambers 2005

Funded by Centro de Tecnologia Canavieira - Piracicaba

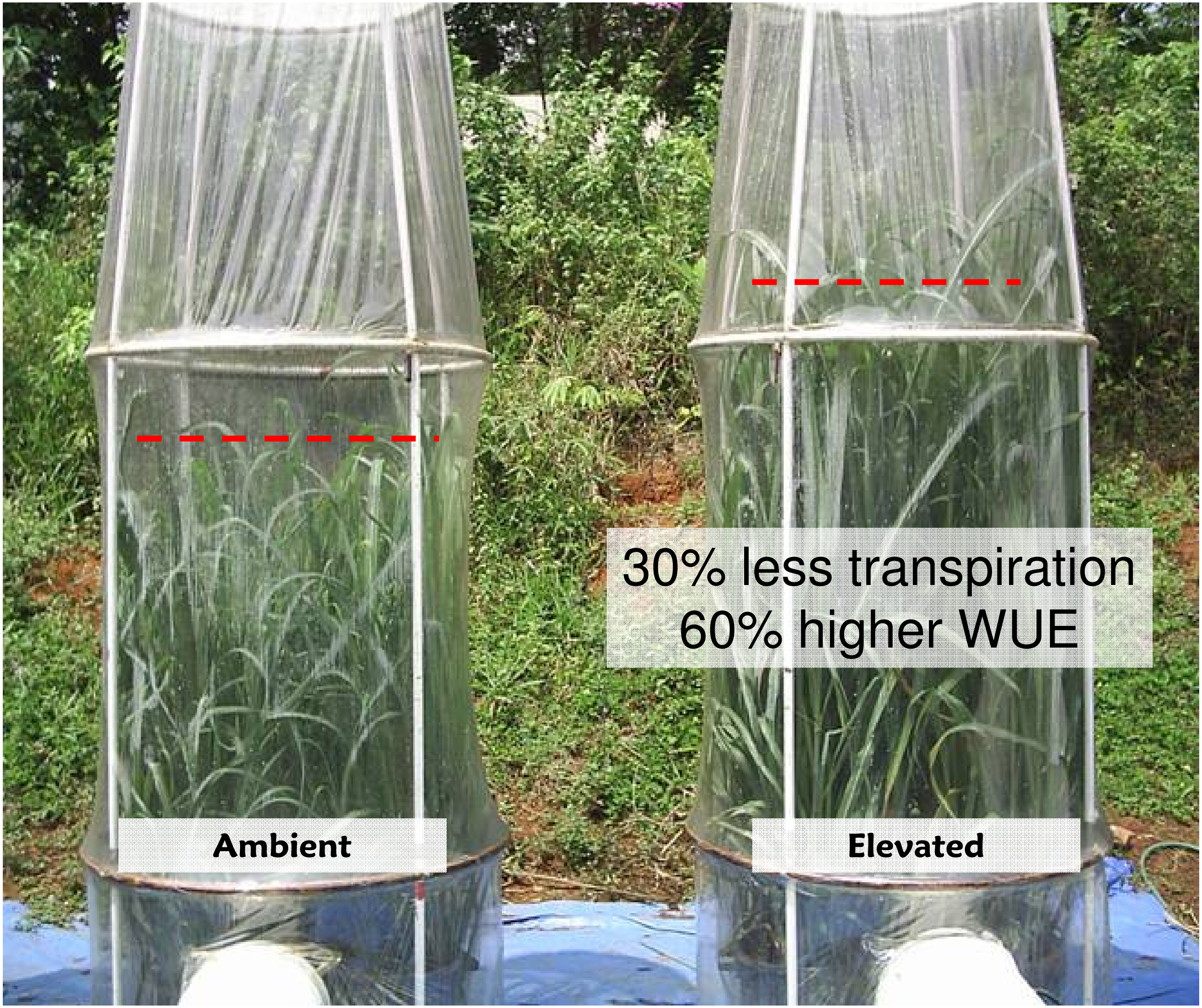
Photosynthesis in sugarcane growing under elevated CO_2

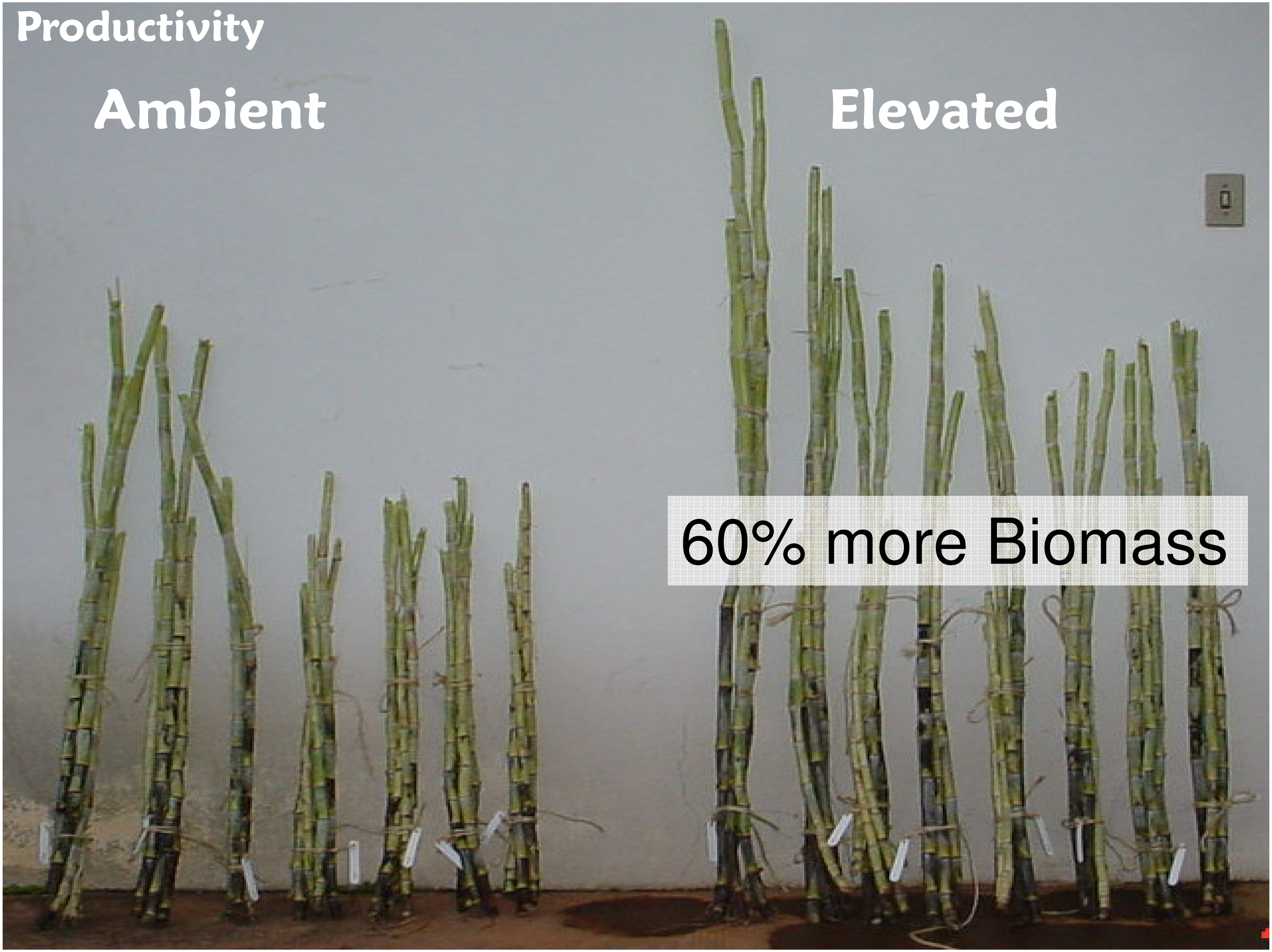


Microarray analysis of the CO_2 experiments

3 months		
Categories	**Gene description**	**Ratio (elevated/ambient)**
Development	light-induced protein	1,194
Photosynthesis	photosystem II protein K; psbK	1,315
Photosynthesis	Ferredoxin I; chloroplast precursor	1,26
Photosynthesis	photosystem I reaction centre subunit n, chloroplast precursor	1,583
Cell wall metabolism	xyloglucan endo-transglycosylase/hydrolase	2,582
Photosynthesis	Chlorophyll A-B binding protein	1,508
Stress response	ASR-like	1,735
Lipid, fatty-acid and isoprenoid metabolism	AE9 stearoyl-ACP desaturase	3,59
Carbohydrate metabolism	beta-glucosidase isozyme 2 precursor	-2,189
Carbohydrate metabolism	putative glucose-6-phosphate dehydrogenase	-1,232
Protein metabolism	translational initiation factor eIF-4A	-1,606

	5 months	
Protein metabolism	large ribosomal protein 2	1,454
Carbohydr. metabolism/Photosynthesis	phosphoenolpyruvate carboxylase	1,245
Cell wall metabolism	Alpha-L-arabinofuranosidase	1,37
Protein metabolism	cathepsin B-like cysteine protease	1,349
Development	dormancy-associated protein	2,299
Transporters	Sugar transporter	1,252
Receptors	serine/threonine-protein kinase NAK	1,706
	unknow	1,271
Protein metabolism	putative glutamate-tRNA ligase	1,504
Protein metabolism	cathepsin B-like cysteine protease	1,395
Protein metabolism	Aldo/keto reductase; Sigma-54 factor	1,229
	putative nucleostemin (GTPase of unknown function)	1,397
Development	putative auxin-independent growth promoter	1,909
Transcription	pre-mRNA splicing factor	1,541
Nucleic acid metabolism	chromodomain-helicase-DNA-binding protein	0,17
Stress response	dehydrin	-1,42
	unknow	-1,398
Secondary metabolism	caffeoyl-CoA 3-O-methyltransferase 1	-1,354
Carbohydrate metabolism	cell wall invertase	-1,615
Stress response	ferritin	-2,768
Protein metabolism	C2 domain-containing protein-like	-1,342
Pathogenicity	Thaumatin	-1,367
Transcription	auxin response factor 2	-1,352
Development	Lateral organ boudaries protein	-1,494
Cell cycle	kelch repeat-containing F-box family protein	-1,541
Cell cycle	cyclin H-1	-1,632


30% less transpiration
60% higher WUE
Ambient
Elevated


Productivity
Ambient
Elevated
60% more Biomass

SUGARS AND FIBERS

	BRIX	Fiber(% FW)	Sucrose (% FW)
Ambient	7.17 ± 0.21	6.62 ± 0.13	2.18 ± 0.20
Elevated	7.75 ± 0.17	7.13 ± 0.21	2.82 ± 0.14*

Speculative calculations

2087 – 653 millions of tons
Etanol – 20 millions of m^3

2050 – 960 millions of tons
Etanol - 32 millions of m^3

However, there are projections for much higher production by 2017

GENES ARE UP, BUT ARE THERE COMPATIBLE BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES?

INCT BIOETANOL

http://bioethanolbrazil.wordpress.com

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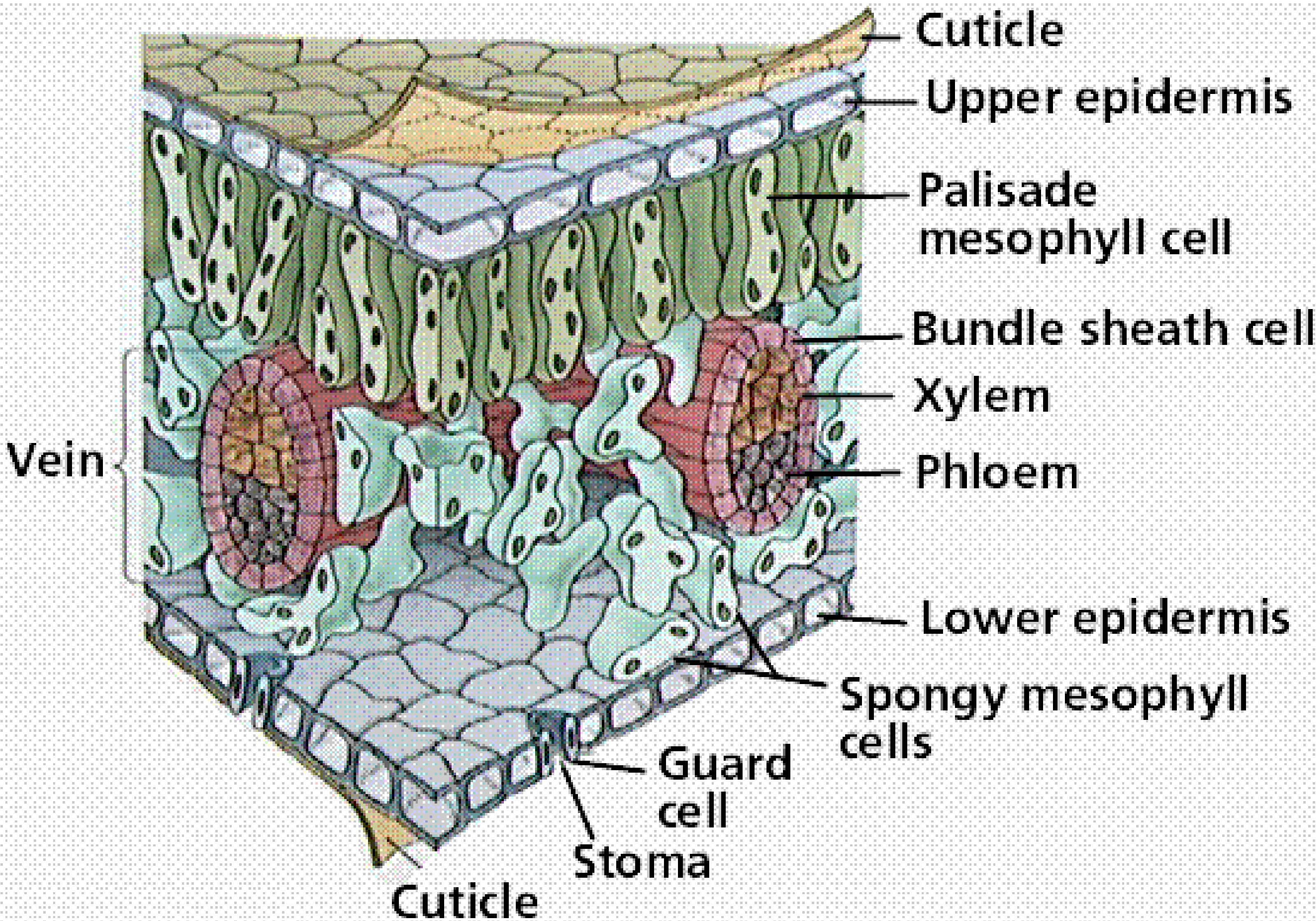
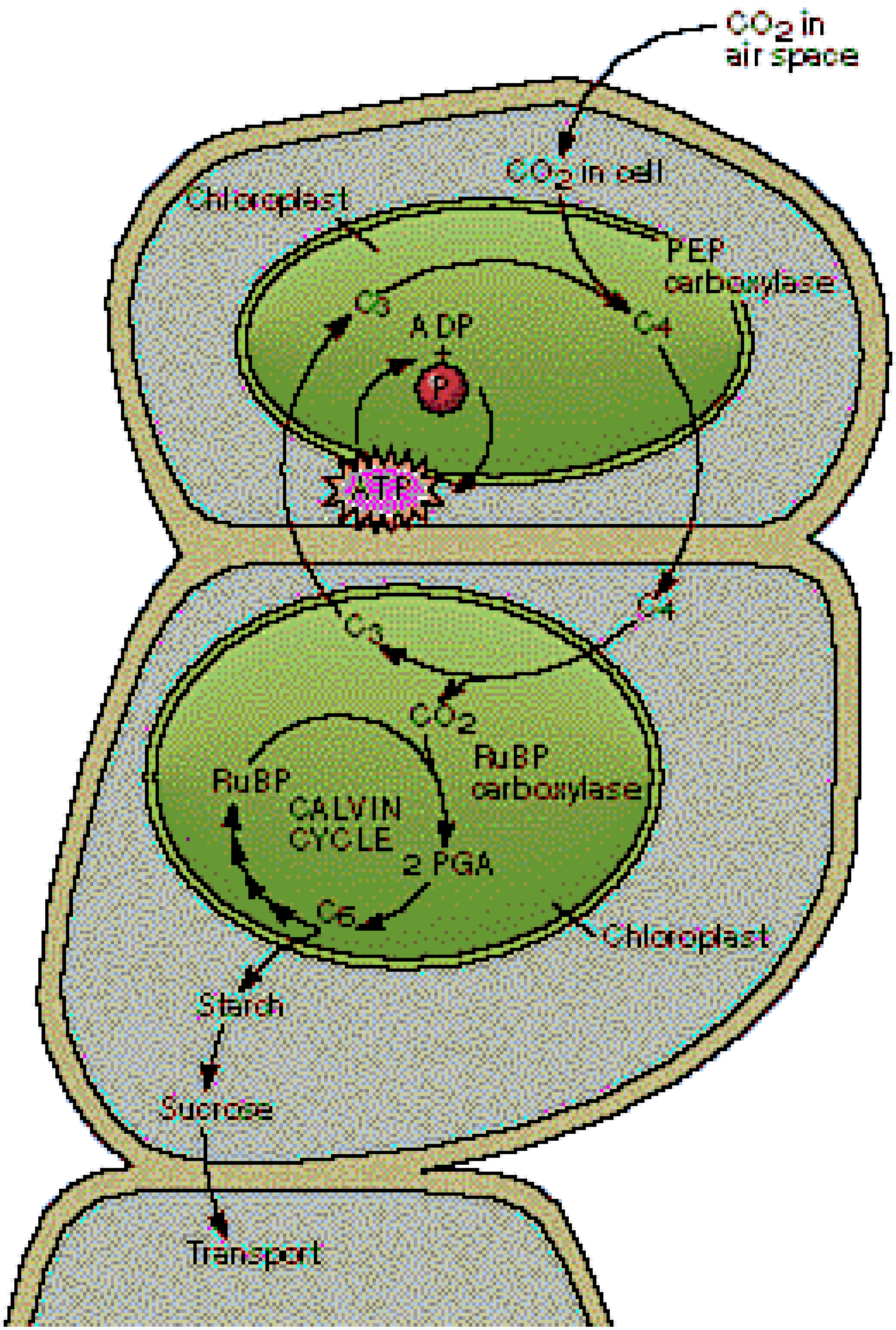
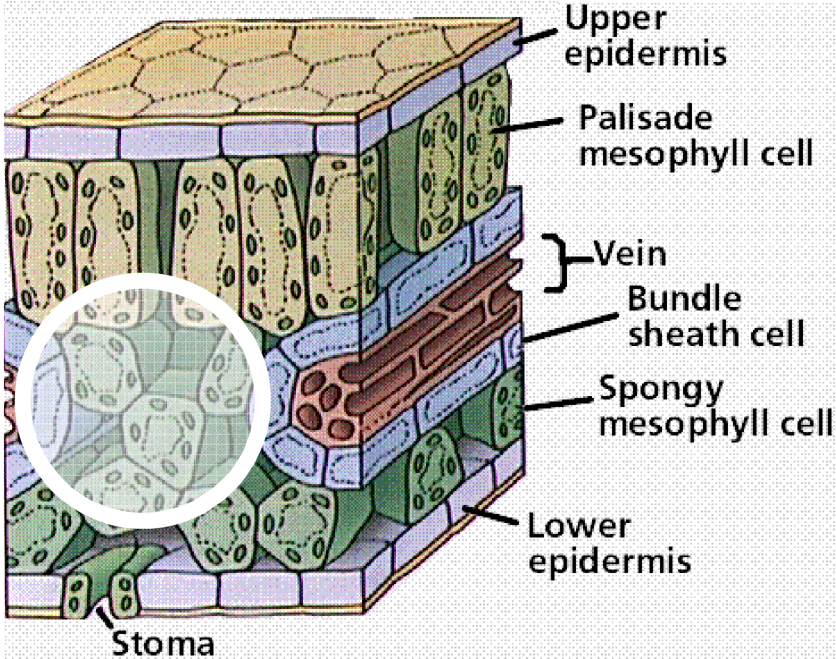
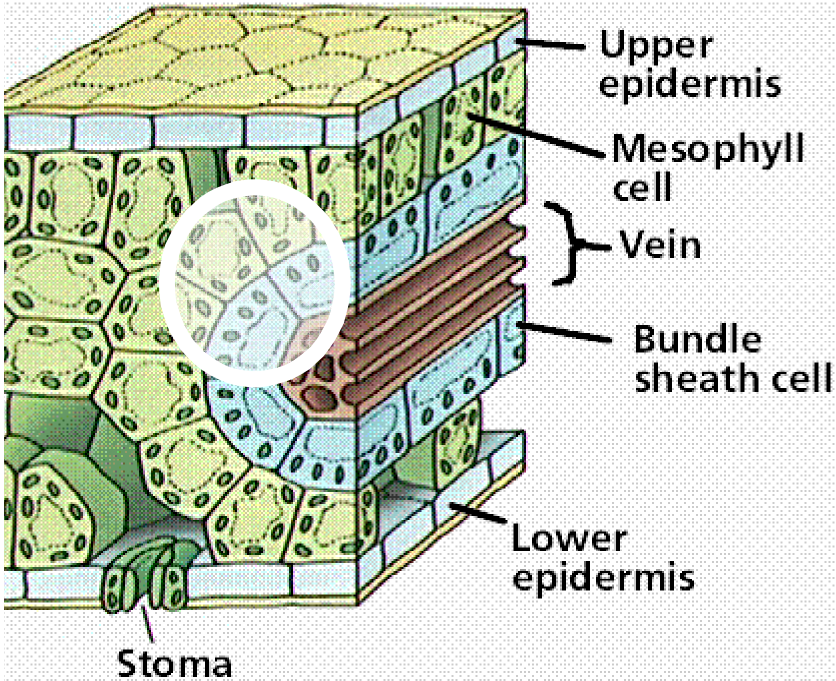
Departamento de Botânica


2008 75 dias em elevado CO_2

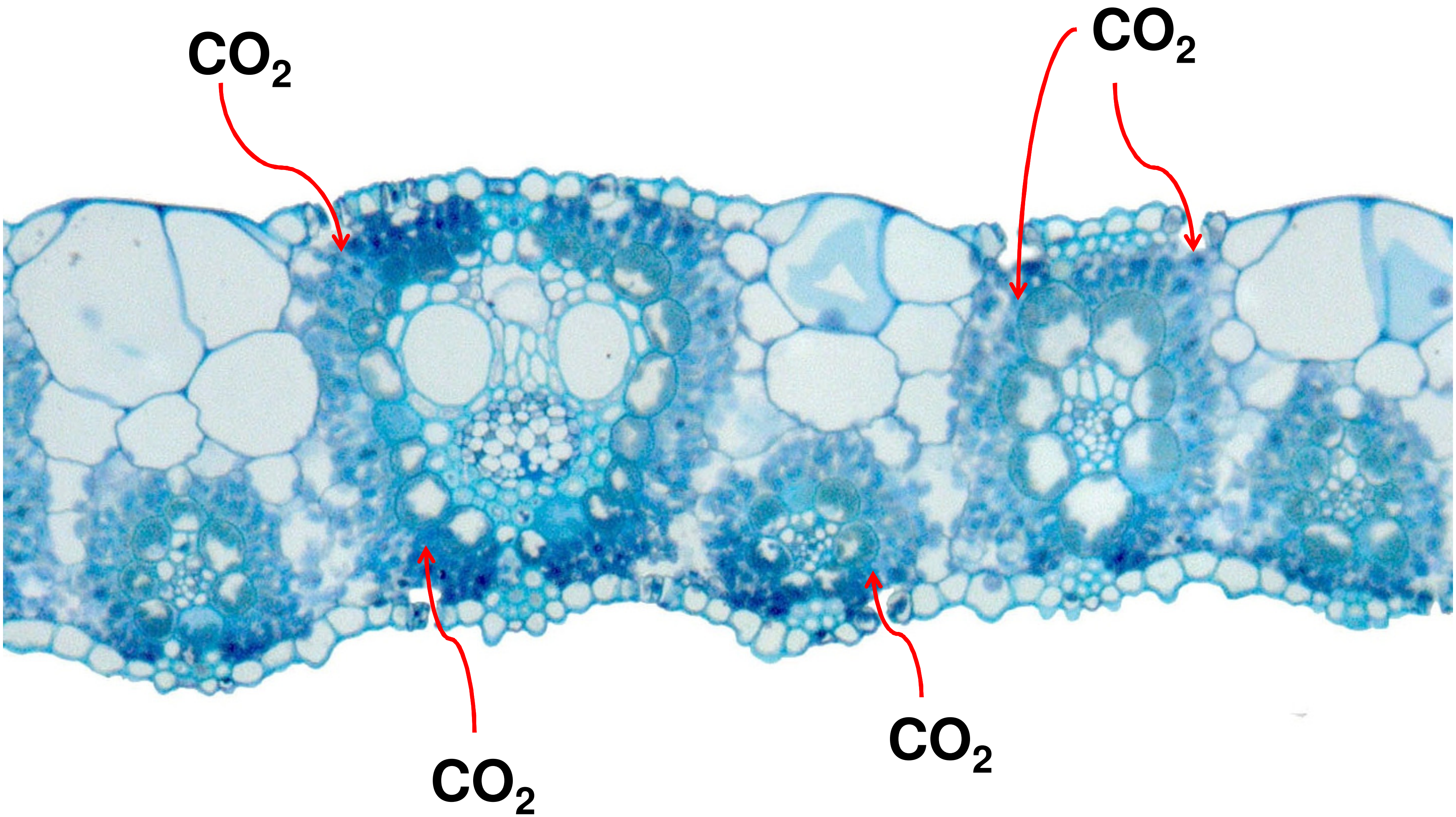

Cuticle
Upper epidermis
Palisade mesophyll cell
Bundle sheath cell
Xylem
Phloem
Vein
Lower epidermis
Spongy mesophyll cells
Guard cell
Stoma
Cuticle


CO_2 in air space
CO_2 in cell
Chloroplast
PEP carboxylase
C3
C4
ADP
P
ATP
C4
C3
CO_2
RuBP
CALVIN CYCLE
RuBP carboxylase
2 PGA
C6
Chloroplast
Starch
Sucrose
Transport


Upper epidermis
Palisade mesophyll cell
Vein
Bundle sheath cell
Spongy mesophyll cell
Lower epidermis
Stoma

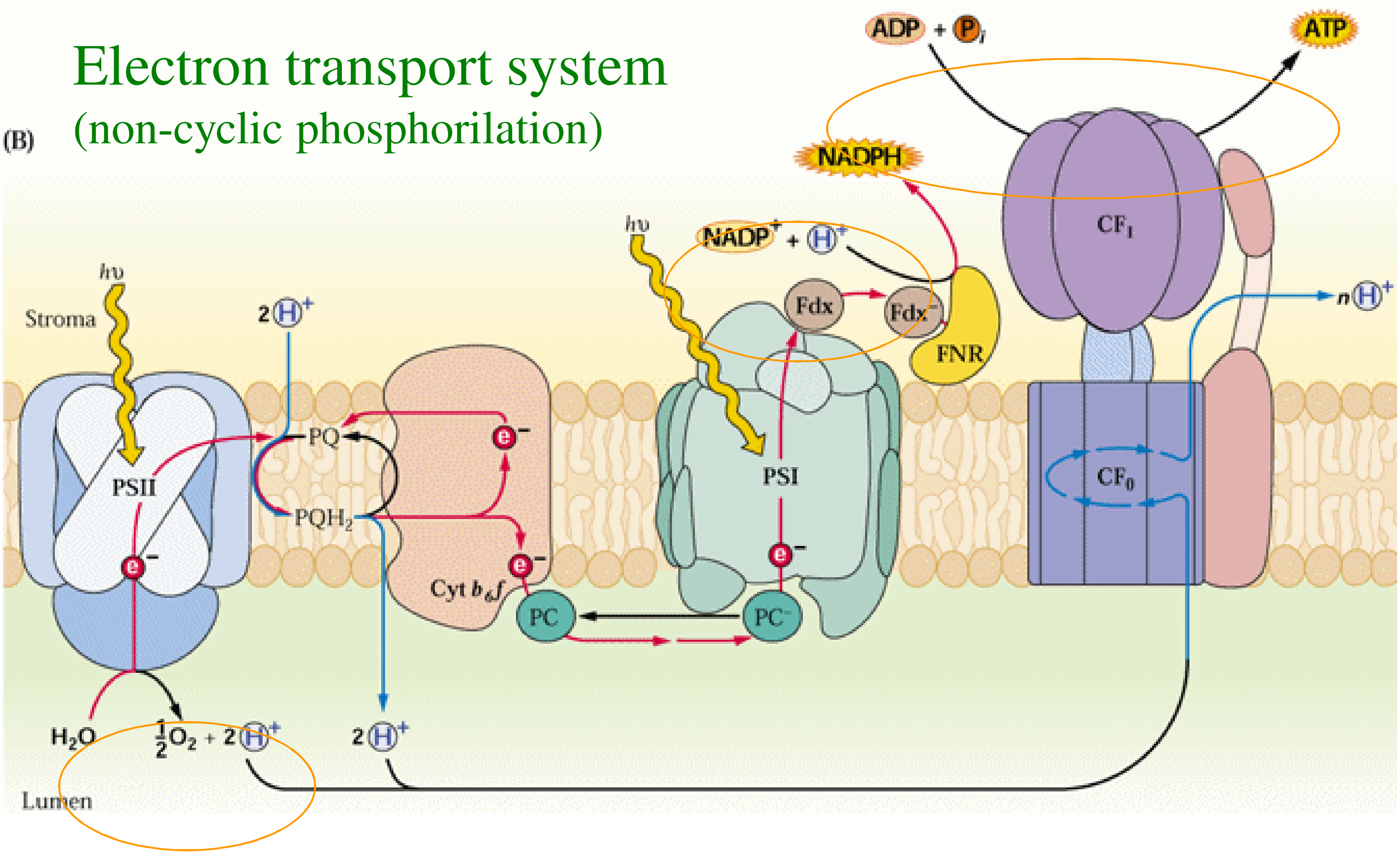

Upper epidermis
Mesophyll cell
Vein
Bundle sheath cell
Lower epidermis
Stoma

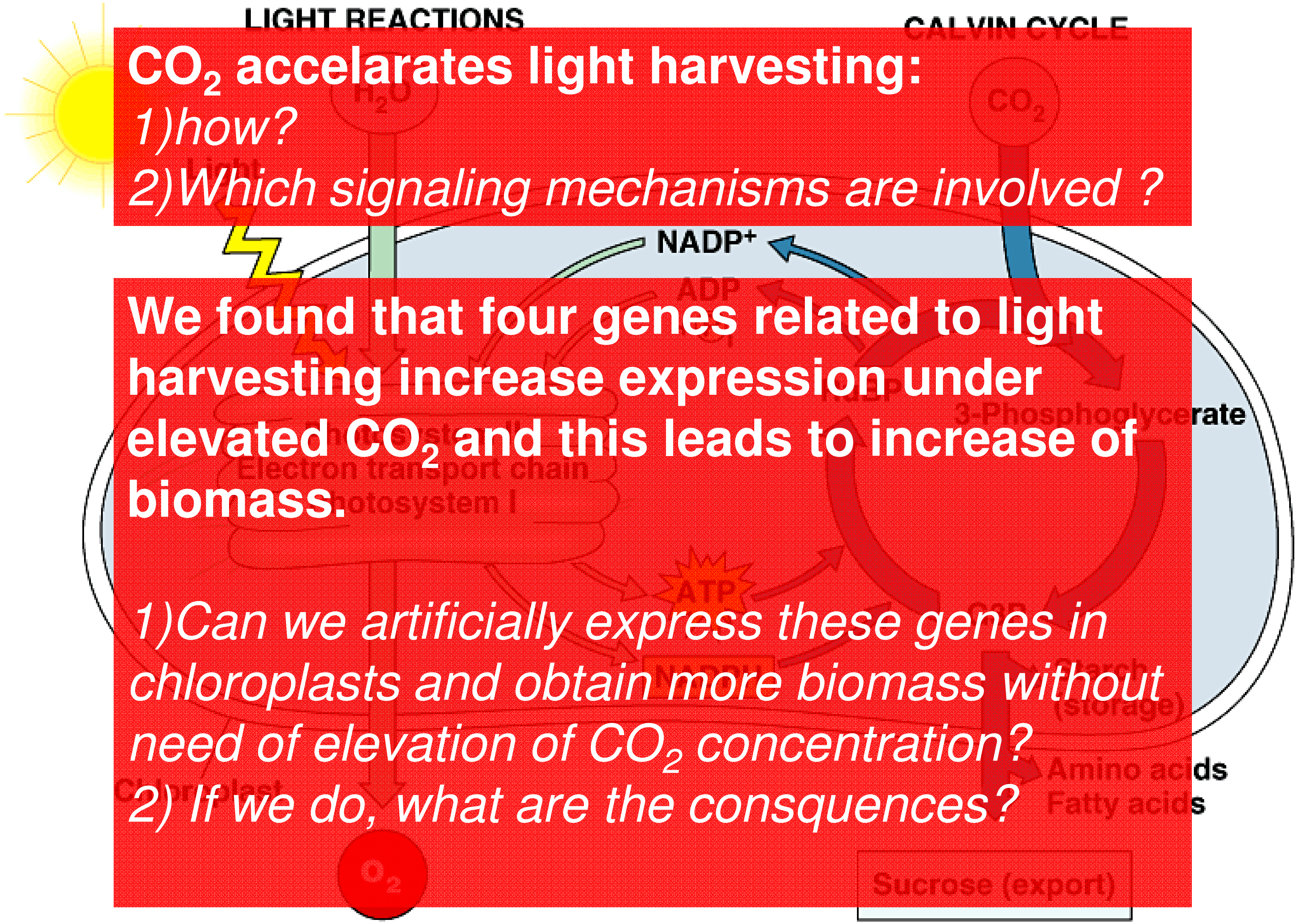
Sugarcane leaves: CO_2 enters at both sides



Electron transport system
(non-cyclic phosphorilation)

(B)





CO_2 accelarates light harvesting:

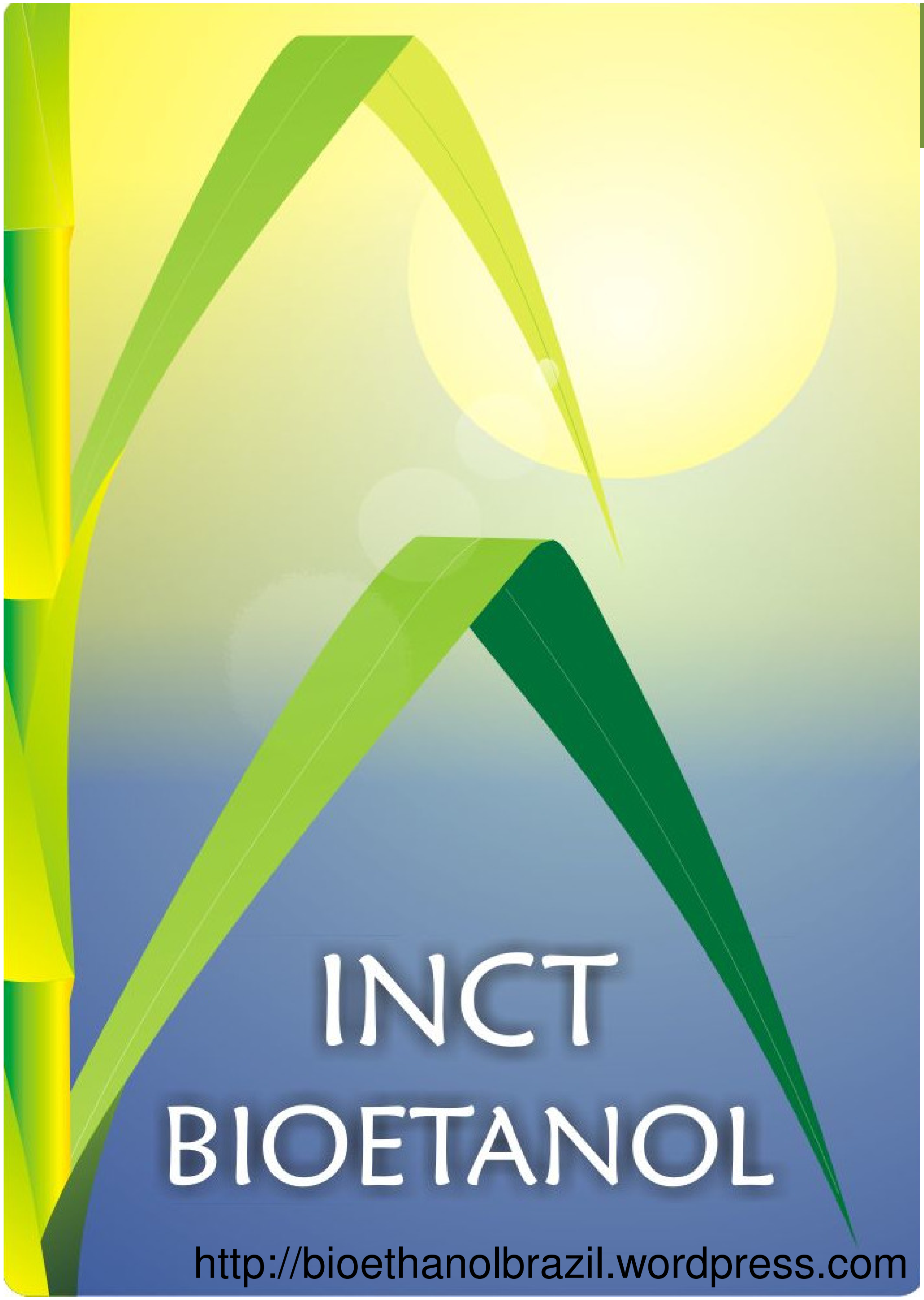
1)how?

2)Which signaling mechanisms are involved ?

We found that four genes related to light harvesting increase expression under elevated CO_2 and this leads to increase of biomass.

1)Can we artificially express these genes in chloroplasts and obtain more biomass without need of elevation of CO_2 concentration?

2) If we do, what are the consquences?



E METABOLISM IN SUGARCANE

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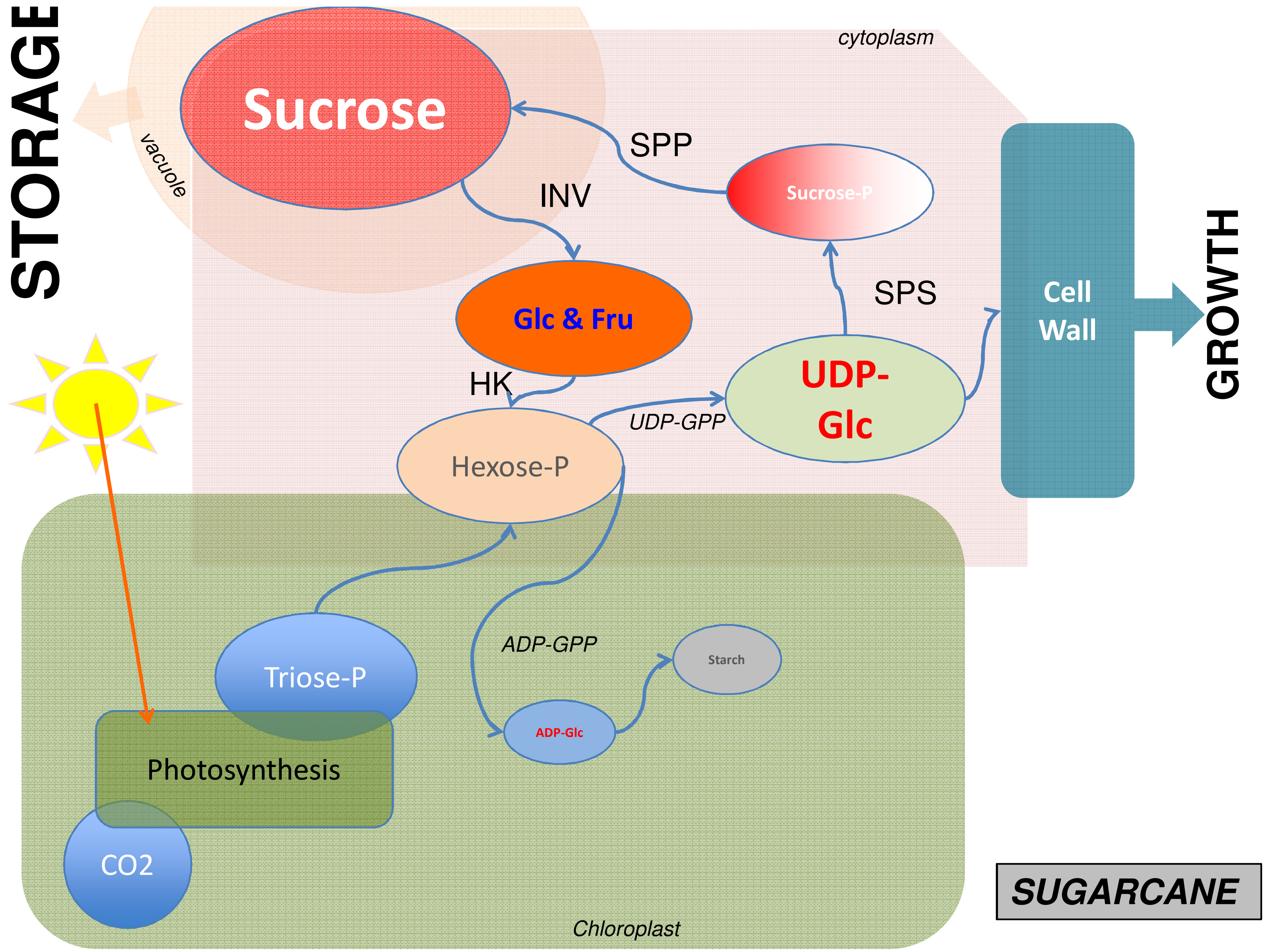
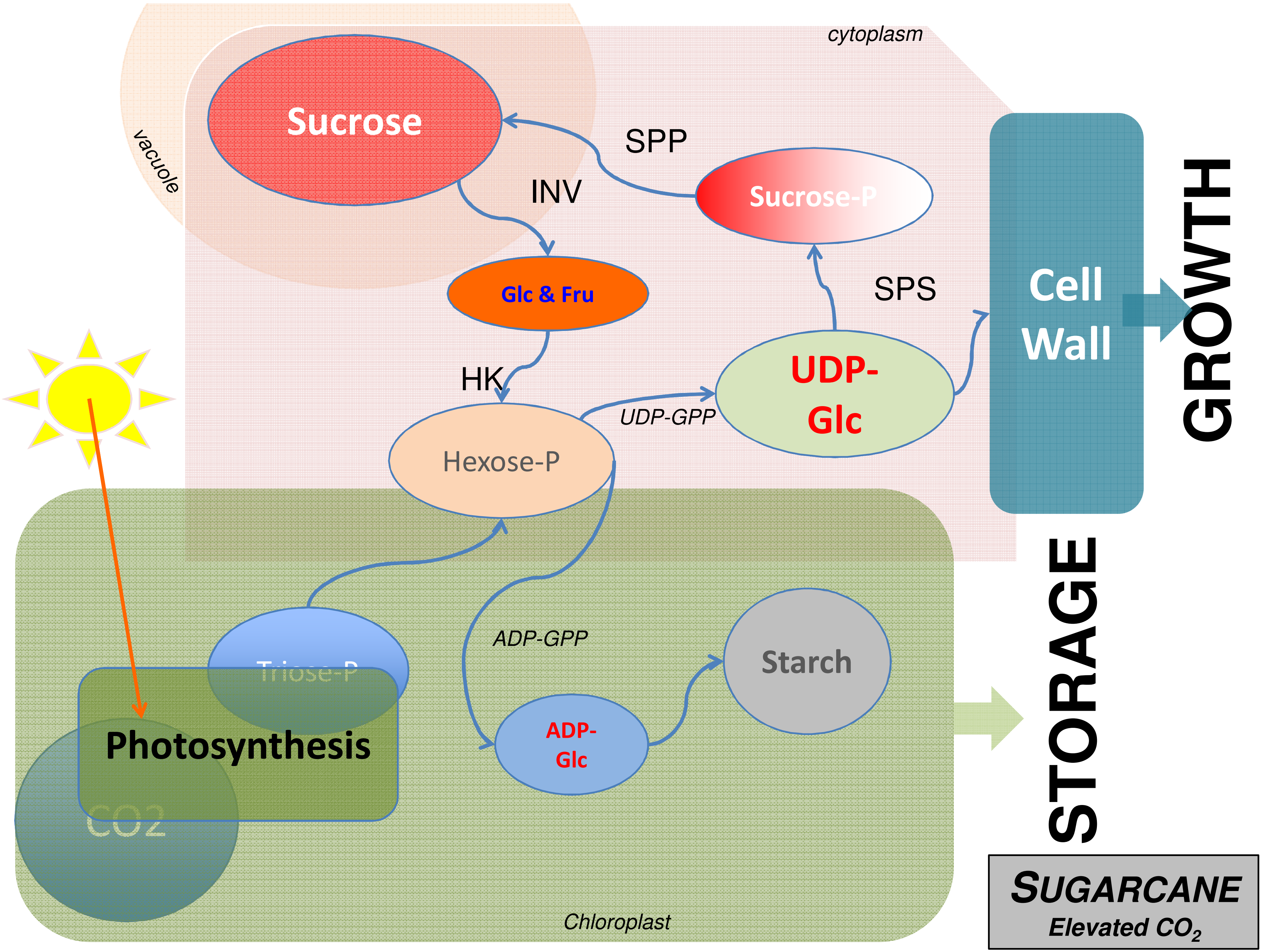
Sugarcane stores sucrose and is hardly capable to produce starch



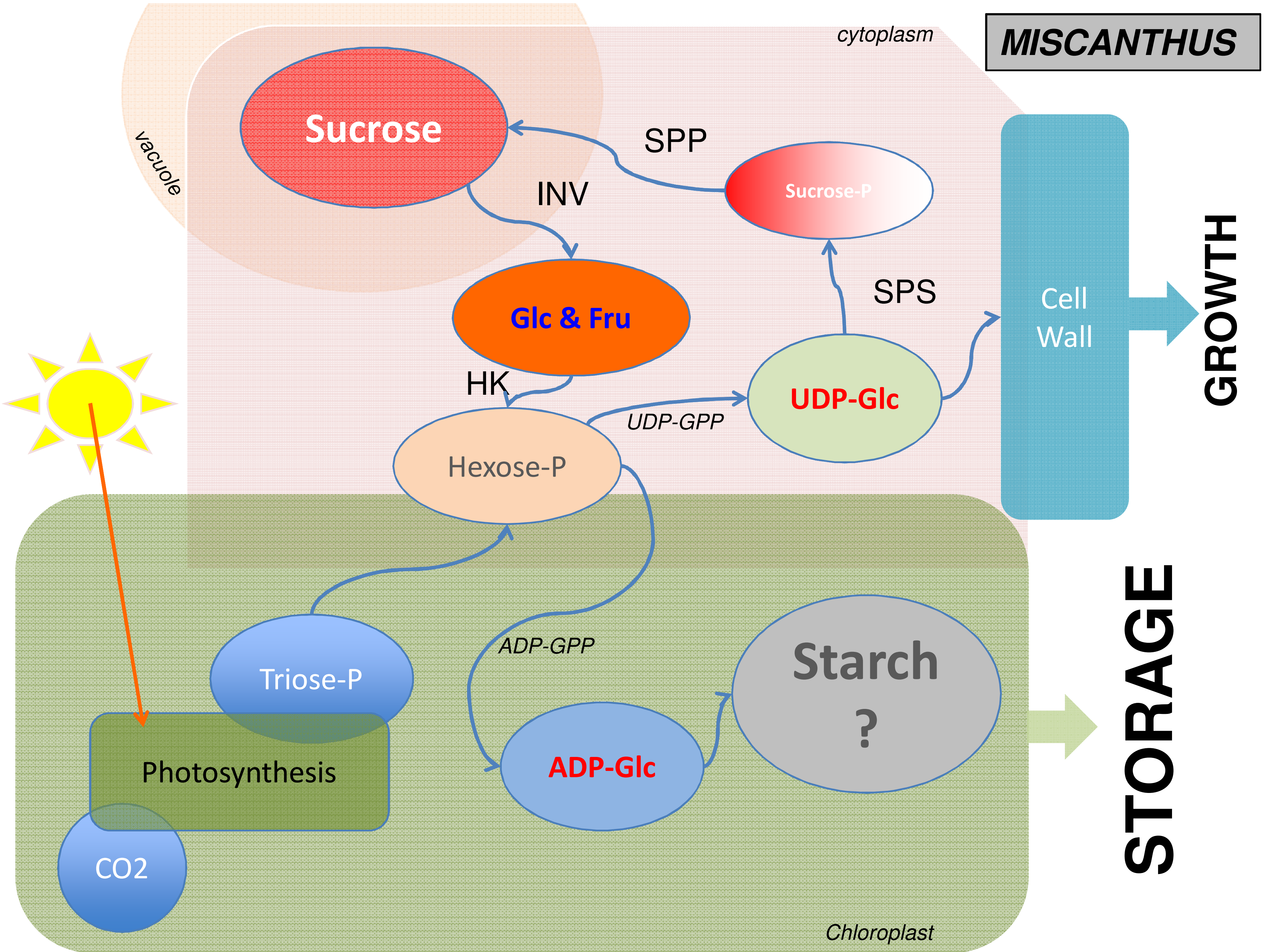
STORAGE
Sucrose
vacuole
cytoplasm
SPP
Sucrose-P
INV
SPS
Glc & Fru
Cell
Wall
GROWTH
HK
UDP-
Glc
UDP-GPP
Hexose-P
ADP-GPP
Starch
Triose-P
ADP-Glc
Photosynthesis
CO2
Chloroplast
SUGARCANE

With elevated CO_2 sugarcane grows more and faster due to increase in ETR, but soluble sugars are lower and there is little starch accumulated in leaves. Instead of the ADP-glc pathway in the chloroplasts, sugarcane leaves seem to use the UDP-glc synthesis to foster cell wall biosynthesis and make more biomass



cytoplasm
vacuole
Sucrose
SPP
INV
Sucrose-P
SPS
Glc & Fru
HK
UDP-GPP
UDP-Glc
Hexose-P
Cell Wall
GROWTH
ADP-GPP
Starch
Triose-P
Photosynthesis
CO2
ADP-Glc
STORAGE
Chloroplast
SUGARCANE
Elevated CO_2

On the other hand, **miscanthus does not accumulate sucrose** as a C storage, as does sugarcane. Thus, it probably uses the UDP-glc pathway to synthesise cell walls and grow but possibly uses the ADP-glc pathway with the same intensity and produces starch in its leaves.



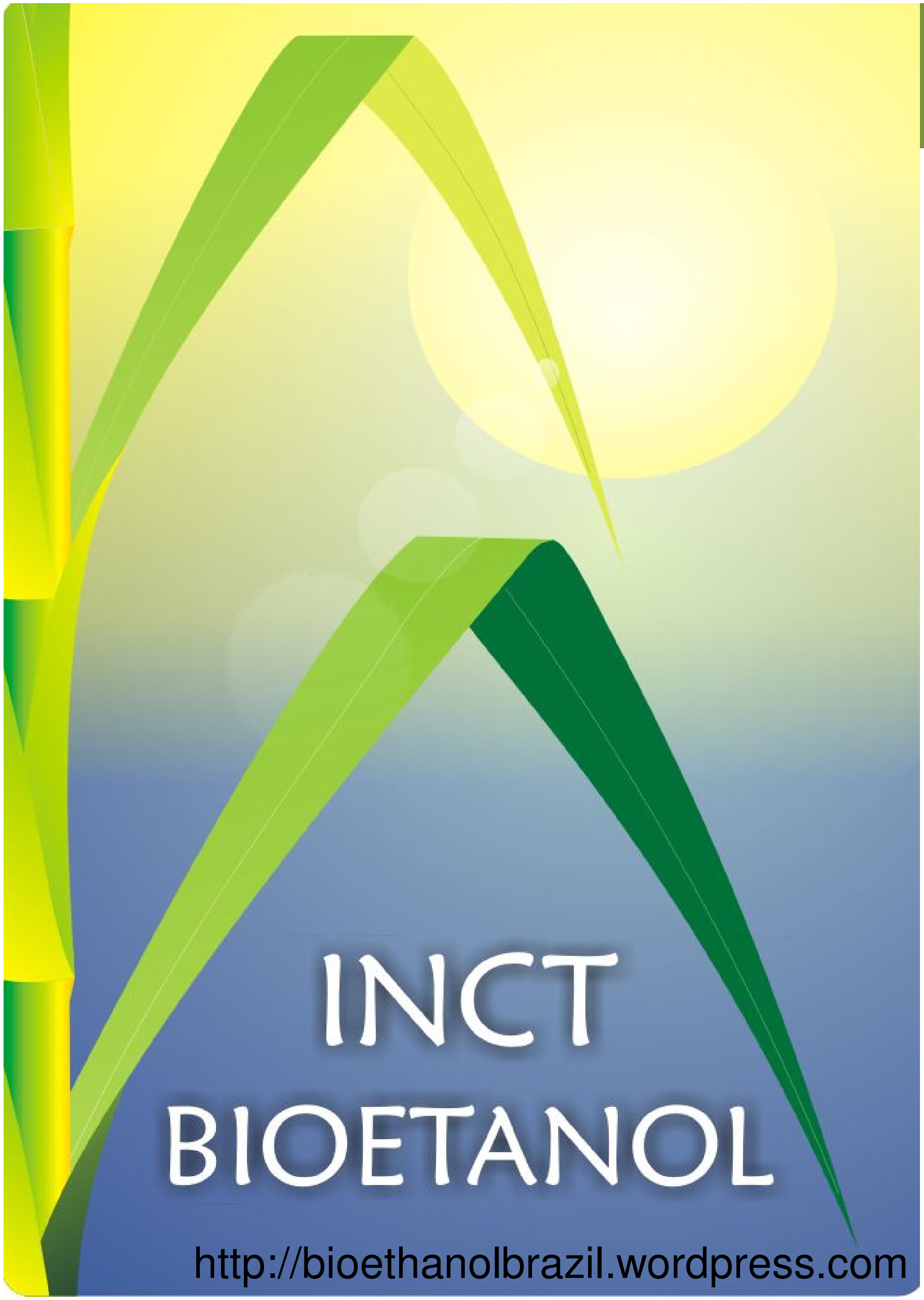
MISCANTHUS
cytoplasm
vacuole
Sucrose
SPP
Sucrose-P
INV
Glc & Fru
SPS
HK
Cell
Wall
GROWTH
UDP-Glc
UDP-GPP
Hexose-P
ADP-GPP
Triose-P
Starch
?
STORAGE
Photosynthesis
ADP-Glc
CO2
Chloroplast

PERSPECTIVES FOR BIOFUELS

A comparison miscanthus X sugarcane would afford to find **network nodes** related with the accumulation of sucrose or cell walls in both species.

This might open the way to **genetically transform each species** so that they could be used either for bioethanol production from sucrose or cellulosic bioethanol

producing starch in sugarcane can me useful as in this way, **more carbon can be "packed"** than when sucrose is stored in vacuoles.



THANK YOU

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BIOEN

FAPESP