South African Sugarcane Research Institute

Gene Discovery:
Approaches, Developments and Applications to Sugarcane Improvement

FAPESP BIOEN
Workshop

18-19 March 2009



OUTLINE

SASRI: Genomics & Functional Genomics Research

- Landscape
- Trajectory

In relation to information, technological resources & research paradigms

Research: Genomics & Functional Genomics Applications

- Marker-Assisted Breeding
- Transgenesis
- Sugarcane Biology

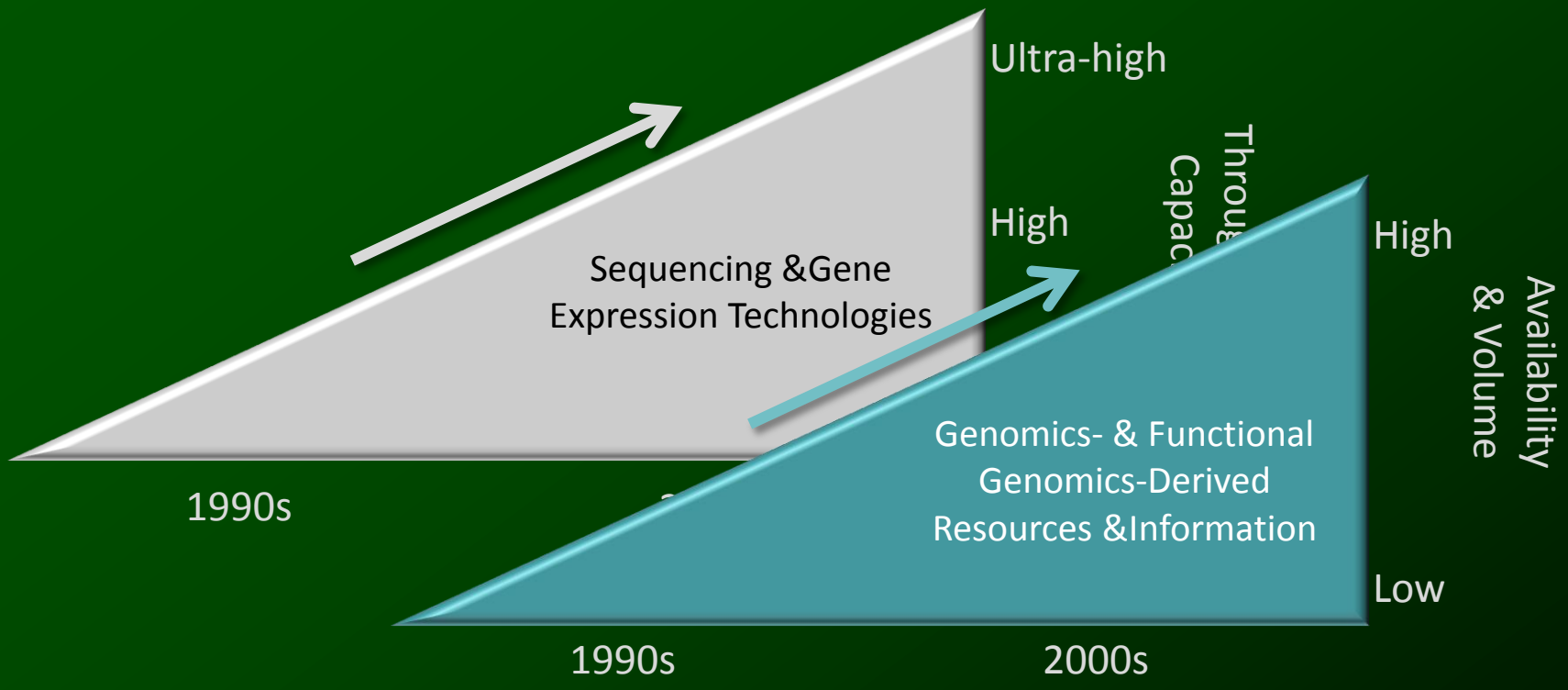
Gene sequences & their expression as access points

Prospects: Genomics & Functional Genomics Advances

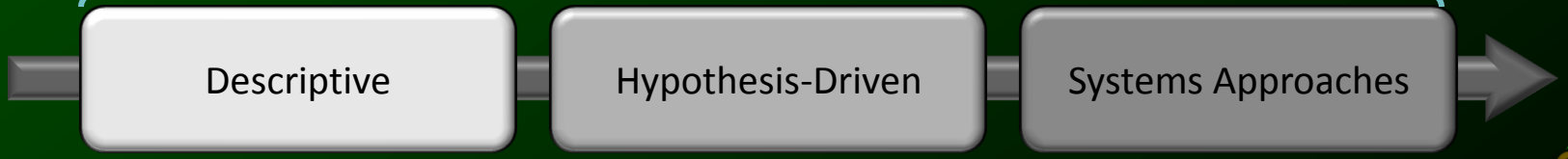
- Marker-Assisted Breeding
- Sugarcane Biology

In relation to emerging technologies & resources

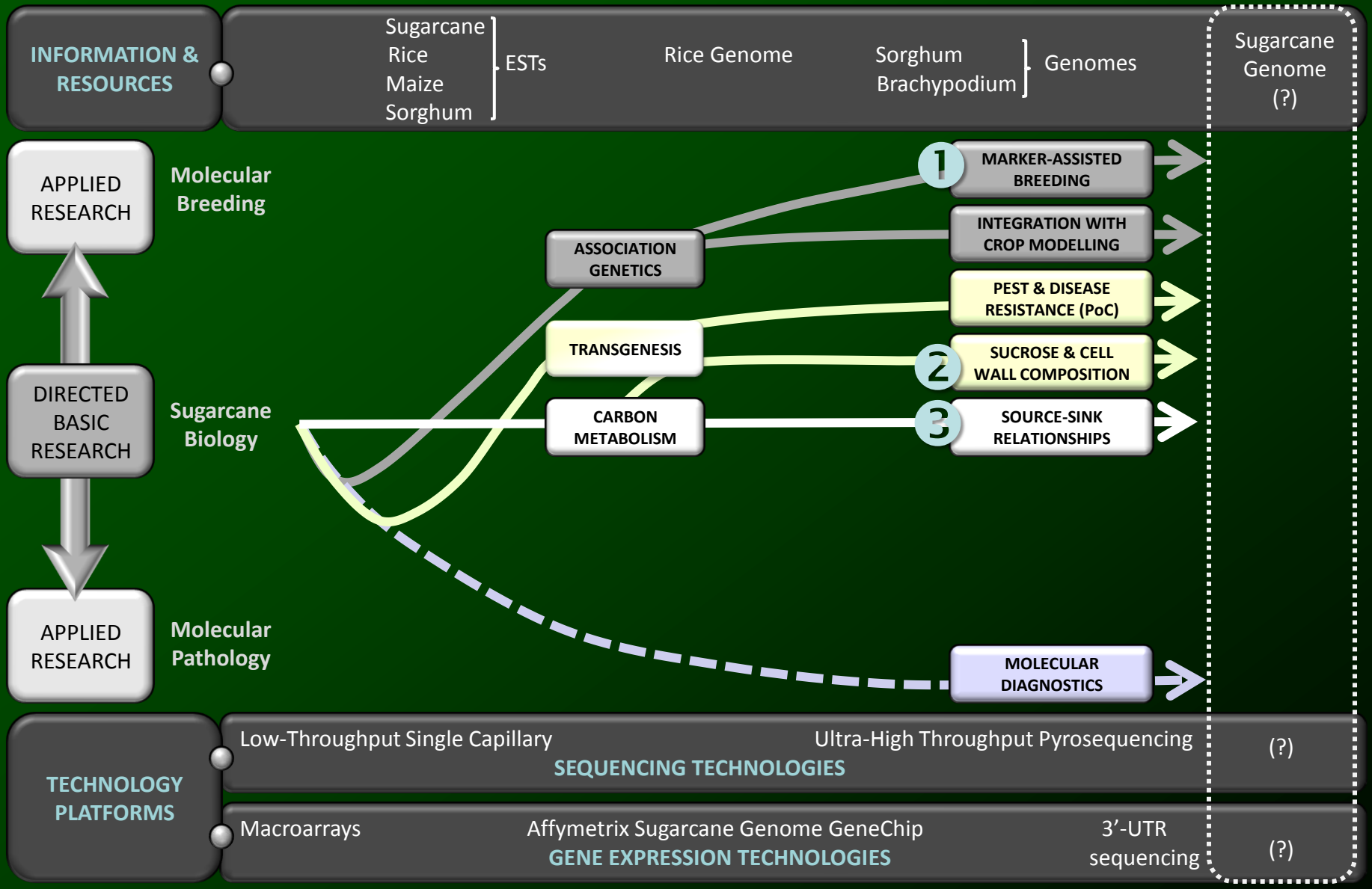
LANDSCAPE: Resources & Research Paradigm



RESEARCH PARADIGMS



TRAJECTORY: From Basic to Applied Research



CURRENT APPLICATIONS:

TECHNOLOGIES & RESOURCES IN GENOMICS & FUNCTIONAL GENOMICS

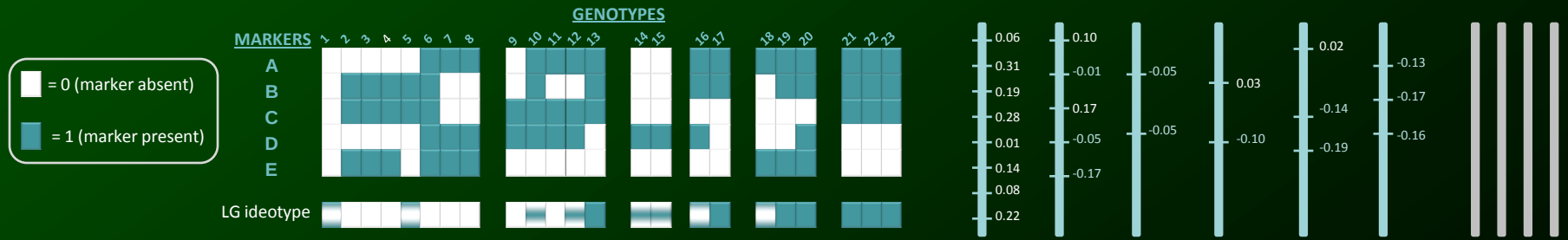
1. MARKER-ASSISTED BREEDING
2. TRANSGENESIS
3. SUGARCANE BIOLOGY (Source-Sink Relationships)

ASSOCIATION GENETICS: MARKER-ASSISTED BREEDING

1 GENE SEQUENCES & THEIR EXPRESSION: MARKER DEVELOPMENT

RATIONALE & APPROACH

ISOLATION:	cDNAs differentially expressed during biotic challenge (potential resistance markers)
	SSH and cDNA-AFLP differential display after pest or disease challenge
IDENTIFICATION:	sequences with putative resistance mechanism function
APPLICATION:	as RFLP probes on a well-characterized Linkage Disequilibrium Mapping population
	numerous anonymous markers (AFLPs, SSRs) also mapped
ANALYSIS:	association mapping to identify markers of resistance/susceptibility



Haplotype Map: Example

ASSOCIATION GENETICS: MARKER-ASSISTED BREEDING

OUTCOMES

- Linkage Disequilibrium Map of 841 markers distributed in 231 haplotypes
- identified haplotypes/markers associated with stalk borer and smut resistance/susceptibility
- markers used to identify cross combinations predicted to give resistant progeny

SASRI: Marker-based crosses for borer and smut resistance

YEAR	NO. CROSSES
2002	79
2003	143
2004	134
2005	78
TOTAL	434

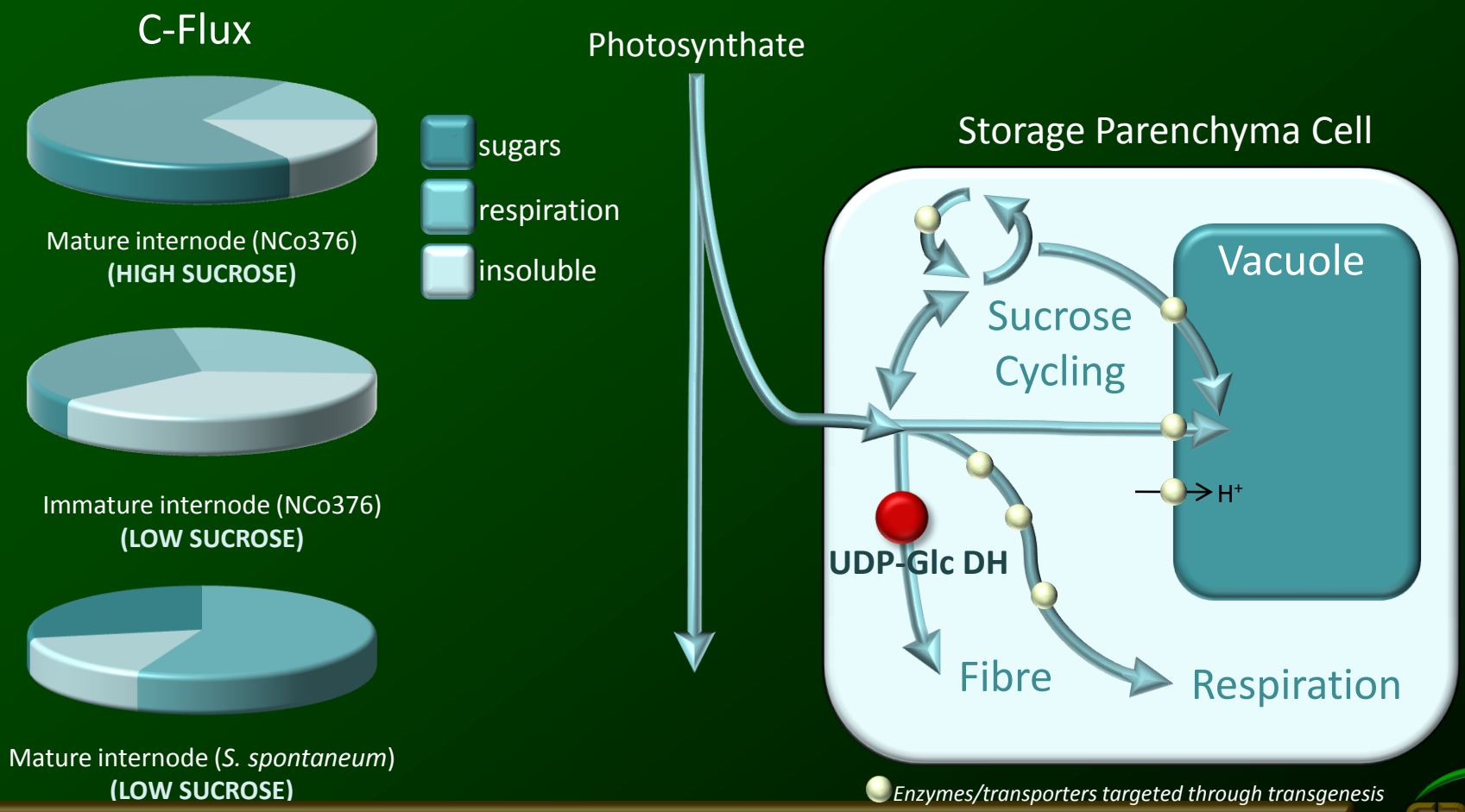
Comparison between heritability for borer resistance calculated from parent phenotype and parent marker-type (Trial of 36 bi-parental families)

	MID-PARENT PHENOTYPE	MARKER PREDICTION
h^2	0.560	0.820
s.e.	0.250	0.280
r^2	0.126	0.192
P-value	0.031	0.007

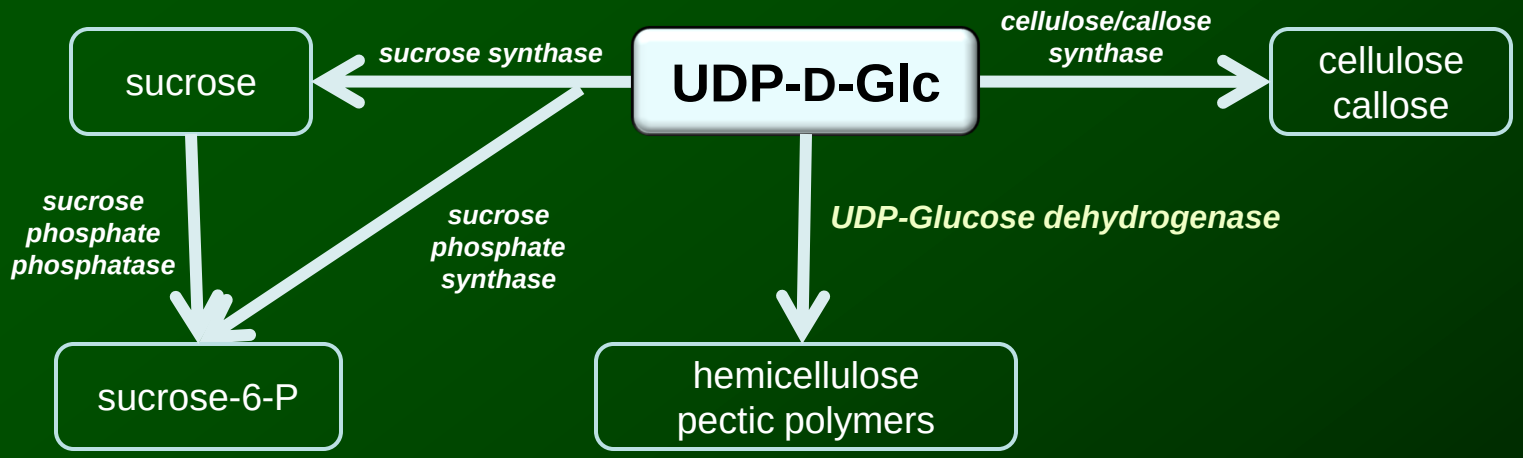
TRANSGENESIS: SUCROSE & CELL WALL

2 GENE SEQUENCES & THEIR EXPRESSION: ACCESSING TRANSGENES

Sugarcane Stalk Physiology



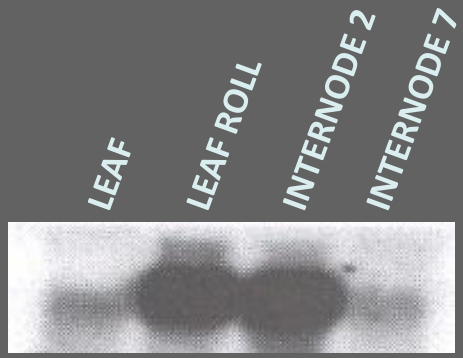
UDP-GLUCOSE: A CENTRAL ROLE IN SUCROSE AND CELL WALL METABOLISM



ESTs Preferentially Expressed in Sugarcane Immature Internodal Tissue. ESTs were isolated by reciprocal **suppression subtractive hybridisations**.

Clone	Sequence homology/ match	Accession Number	Sequence Identity (%)	*E* Value
I2-6	*Glycine max* UDP-glucose dehydrogenase	U53418	78	9.7×10^{-44}
I2-7	*G. max* UDP-glucose dehydrogenase	U53418	92	4.2×10^{-54}
I2-13	*G. max* UDP-glucose dehydrogenase	U53418	96	1.4×10^{-9}
I2-155	*G. max* UDP-glucose dehydrogenase	T08818	90	2.0×10^{-43}
I2-160	*G. max* UDP-glucose dehydrogenase	Q96558	95	1.0×10^{-39}

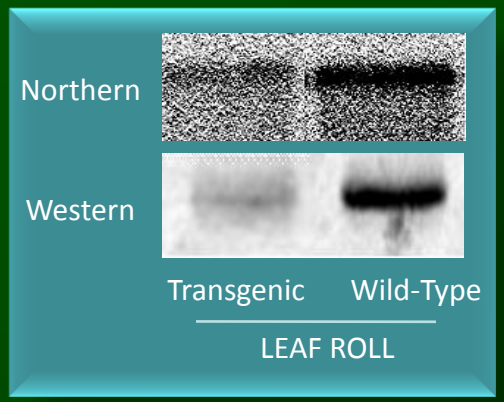
Expression of UDP-Glc DH Clone I2-160 in *Saccharum* spp hybrid NCo376

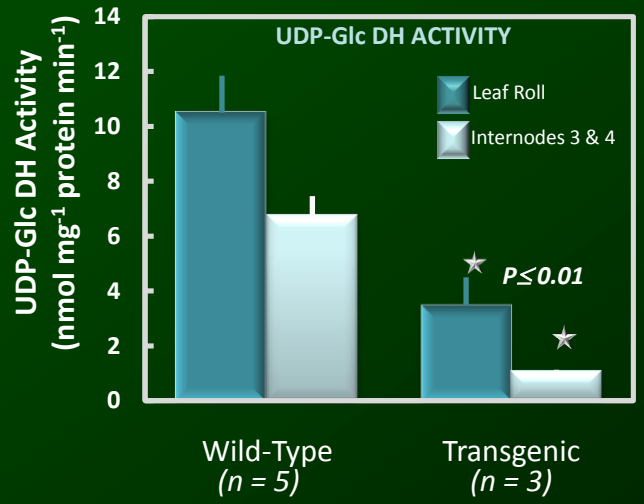


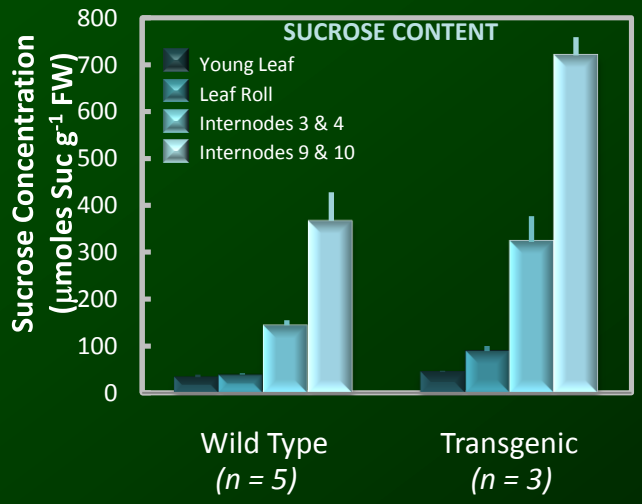
EST Profiling & SSH: Provided Direction and Genetic Resources

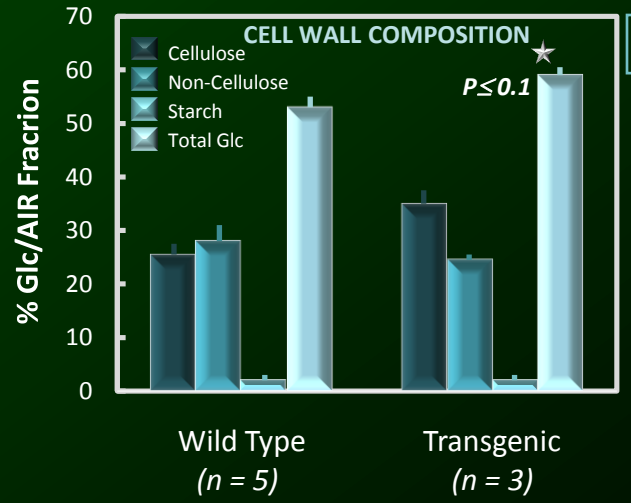
TARGETTING SUCROSE AND CELL WALL METABOLISM

Down-Regulation of UDP-Glucose Dehydrogenase (Greenhouse Trials)









Internodes 9 + 10

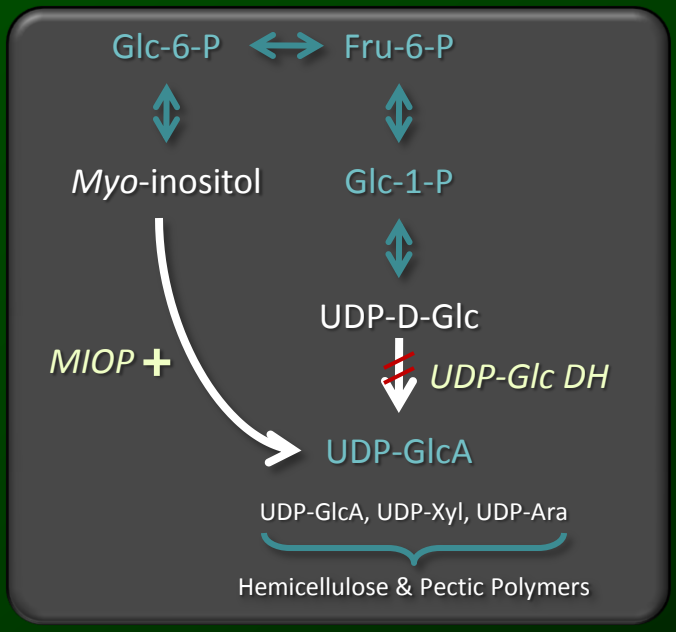
TARGETING SUCROSE AND CELL WALL METABOLISM

Down-Regulation of UDP-Glucose Dehydrogenase (Greenhouse Trials)

Repression of UDP-Glc DH Activity

- ↑ [Uronic Acid]
- ↑ [Total Glc] & Cellulose
- ↑ Arabinose : Galactose
- ↑ Pentose : Hexose (NS)
- ↑ Xylose : Galactose (NS)

Compensatory ↑ in *Myo*-Inositol Oxygenase Activity



Validation Under Field Conditions in Progress

Lessons Learned:

- Confirmation of Plasticity of Primary Metabolism
- Necessity of Exploring Changes in Surrounding Metabolism

SUGARCANE BIOLOGY: SOURCE-SINK RELATIONSHIPS

3 GENE SEQUENCES & THEIR EXPRESSION: UNDERSTANDING SYSTEMS

Physiological processes that may participate in regulating sucrose accumulation

LEAF	Leaf photosynthetic rates & photosynthate partitioning/sequestration
TRANSPORT	Phloem loading & unloading (& leakage & recapture during transport)
STALK	Membrane transport (cell membrane, tonoplast)
	Sucrose turnover (cycling, remobilization)
	Allocation (glycolytic flux, fibre biosynthesis)
NEW FOCUS	Sensitivity of source photosynthetic activity to sink demand

Evidence for supply-demand driven regulation of photosynthesis in sugarcane:

End product repression of photosynthesis in sugarcane:

(Hartt & Burr, 1967; Waldron *et al*., 1967 Bull & Tovey, 1974; Irvine, 1975; Amaya *et al*., 1995; Allison *et al*., 1997)

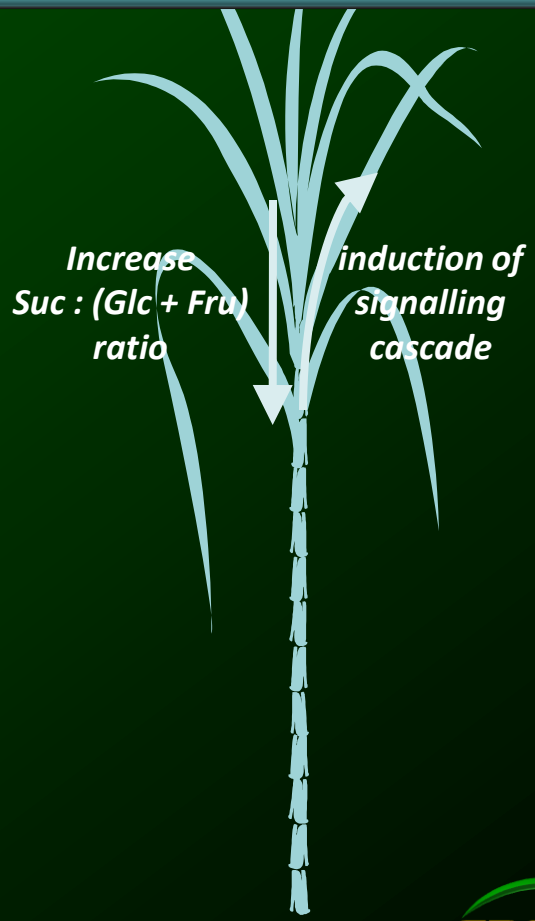
- Down-regulation of photosynthesis
- Induction of leaf physiological decline (senescence)

Evidence of photosynthetic 'plasticity' in sugarcane:

(Wu & Birch, 2007)

Well-documented evidence of source-sink feedback in other plants:

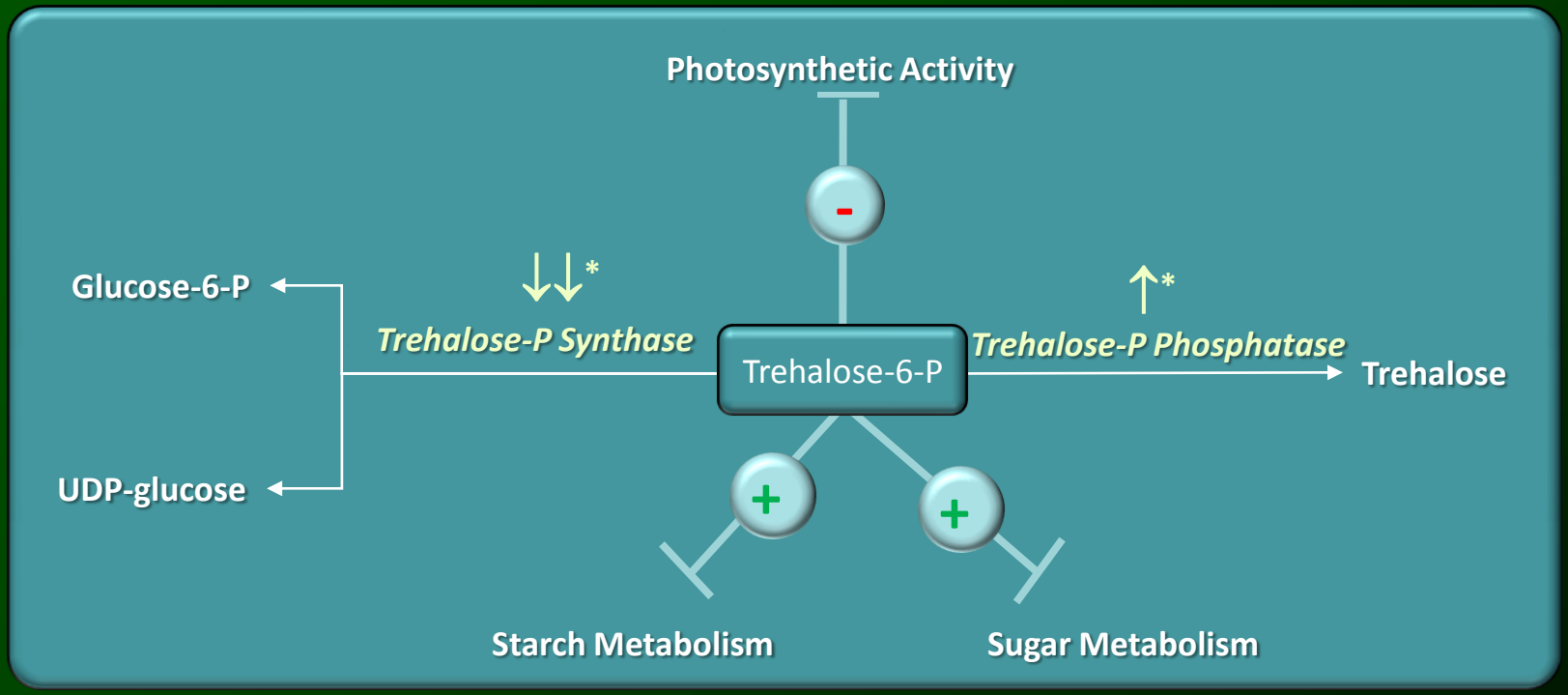
(Paul & Foyer, 2001; Paul & Pellny, 2003; Rolland *et al*., 2006; Paul, 2007)



Analysis of Potential Signalling Mechanism: Affymetrix Sugarcane Genome GeneChip

UP-REGULATED			DOWN-REGULATED		
Putative Gene Identity	**Fold Change**		**Putative Gene Identity**	**Fold Change**	
Photosynthesis					
			ATP synthase CF0 C Chain	0.20	↓↓
			ATP synthase CF1 beta chain	0.30	↓↓
			Cytochrome P540	0.10	↓↓↓
			Photosystem I subunit O	0.05	↓↓↓
			Phosphoglycerate kinase (chloroplastic)	0.30	↓↓
			RuBisCO large subunit-binding protein	0.40	↓
			RuBisCO small subunit	0.10	↓↓↓
Sugar signalling, transport & P_i metabolism					
Glucose-phosphate/phosphate transporter	9.7	↑↑↑	Trehalose-6-phosphate synthase	0.30	↓↓
Trehalose-phosphatase	3.6	↑			
Inorganic pyrophosphatase	4.4	↑↑			
Phosphate translocator	4.4	↑↑			
Starch metabolism					
1,4-α-glucan branching enzyme	2.0	↑			
ADP-glucose pyrophosphorylase	12.8	↑↑↑			
β-amylase	5.2	↑↑			
Isoamylase	8.2	↑↑↑			
Phosphorylase	8.8	↑↑↑			

Potential Central Role of Trehalose-6-Phosphate in Source-Sink Signalling



* As per Affymetrix Sugarcane Genome GeneChip analysis

PROSPECTS:

APPLICATIONS OF NEW TECHNOLOGIES & RESOURCES IN GENOMICS & FUNCTIONAL GENOMICS

- MARKER-ASSISTED BREEDING
- SUGARCANE BIOLOGY

  (Source-Sink Relationships)

MARKER-ASSISTED BREEDING : CURRENT RESEARCH

ICSB Projects

- LD Map of SA breeding population
- Identification of QTAs for yield component traits

Project 21

- Extended LD Map of SA population (DArT)
- Extended reference genetic map of R570 population

Project 26

R570 REFERENCE MAP

MARKER	NUMBER
RFLP	211
SSR	140
AFLP	689
DArT	890
TOTAL	1930

LD MAP

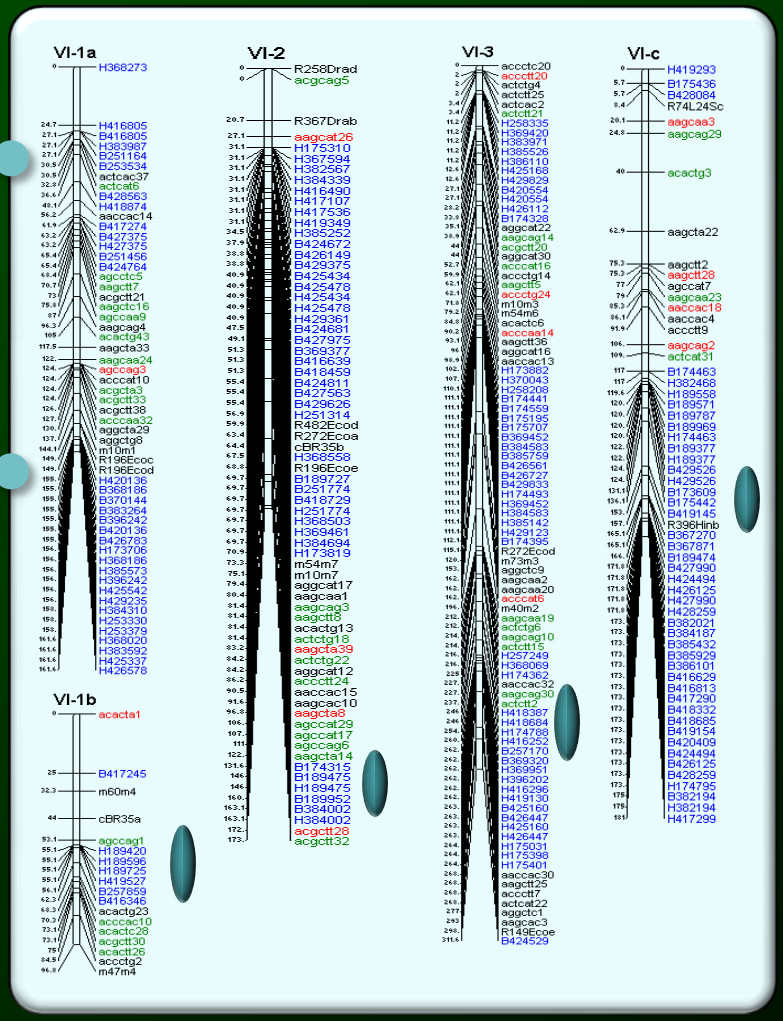
MARKER	NUMBER
AFLP	737
DArT	1906
Unique DArT	1263
Unique Markers	890

373 DArT markers common to both maps

MARKER-ASSISTED BREEDING : CURRENT RESEARCH

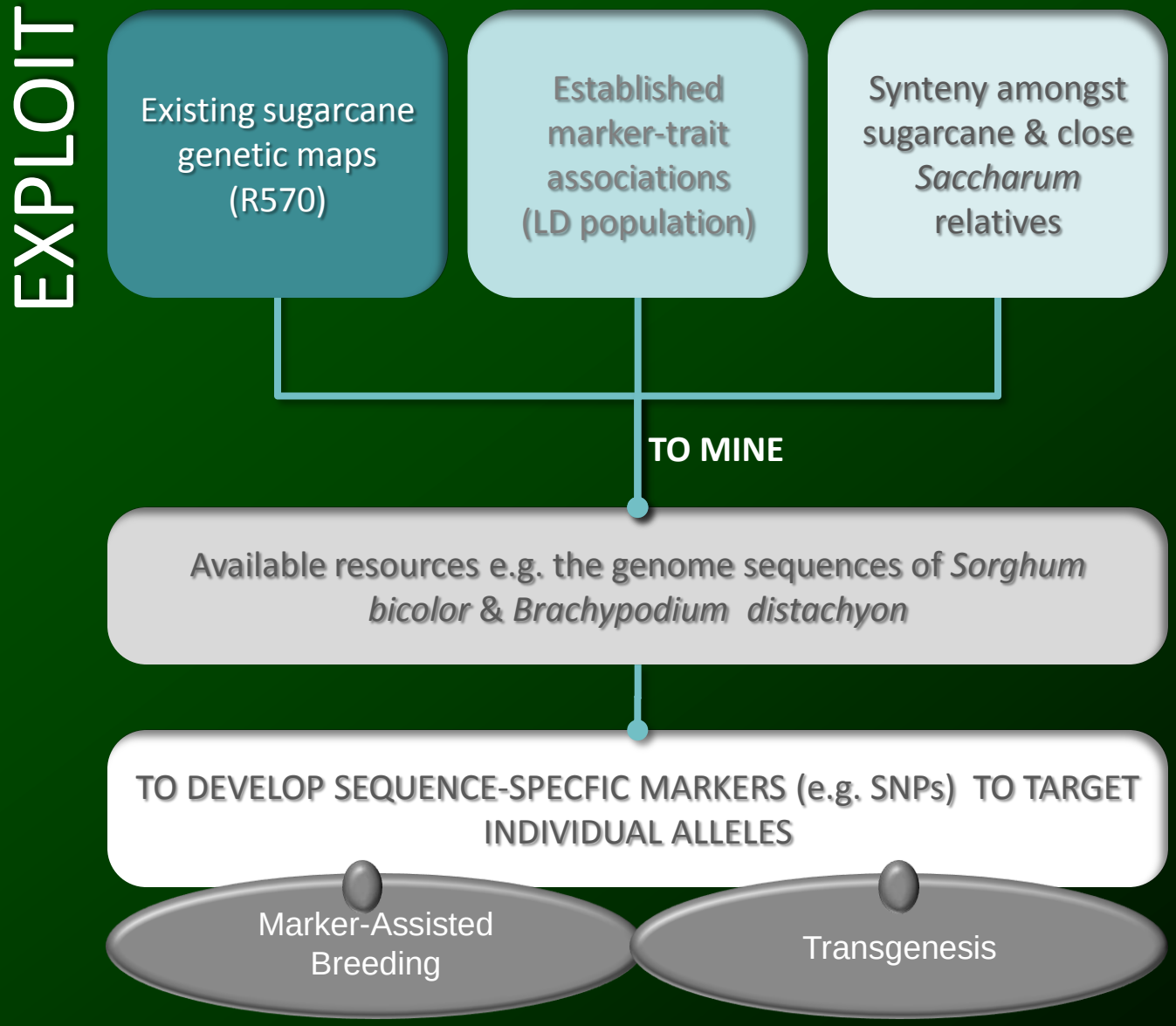
Established position of markers from the LD map on the genetic map of R570

Identified potential QTLs for cane yield from LD Map



Research Outcomes : Drs Mike Butterfield *et al.* (ICSB Projects 21 & 26)

MARKER-ASSISTED BREEDING : PROSPECTS



SUGARCANE BIOLOGY (SOURCE-SINK RELATIONSHIPS) : CURRENT RESEARCH

GOAL

To deploy high-throughput technologies and bioinformatic resources to determine the molecular mechanism underlying the sensitivity of photosynthetic carbon metabolism and related metabolic pathways to fluctuating sugar concentrations in the leaves of sugarcane.

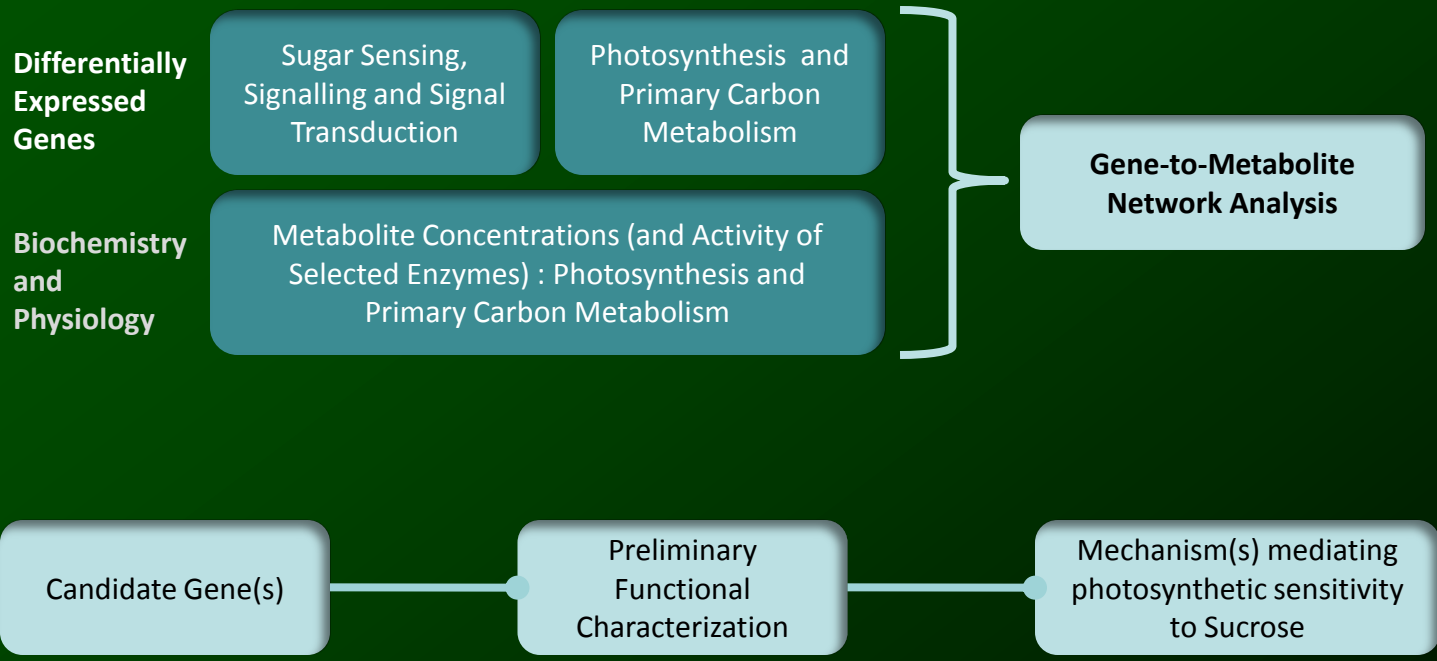
APPROACH

Artificially perturbed leaf sugar status (simulated effect of internode maturation)

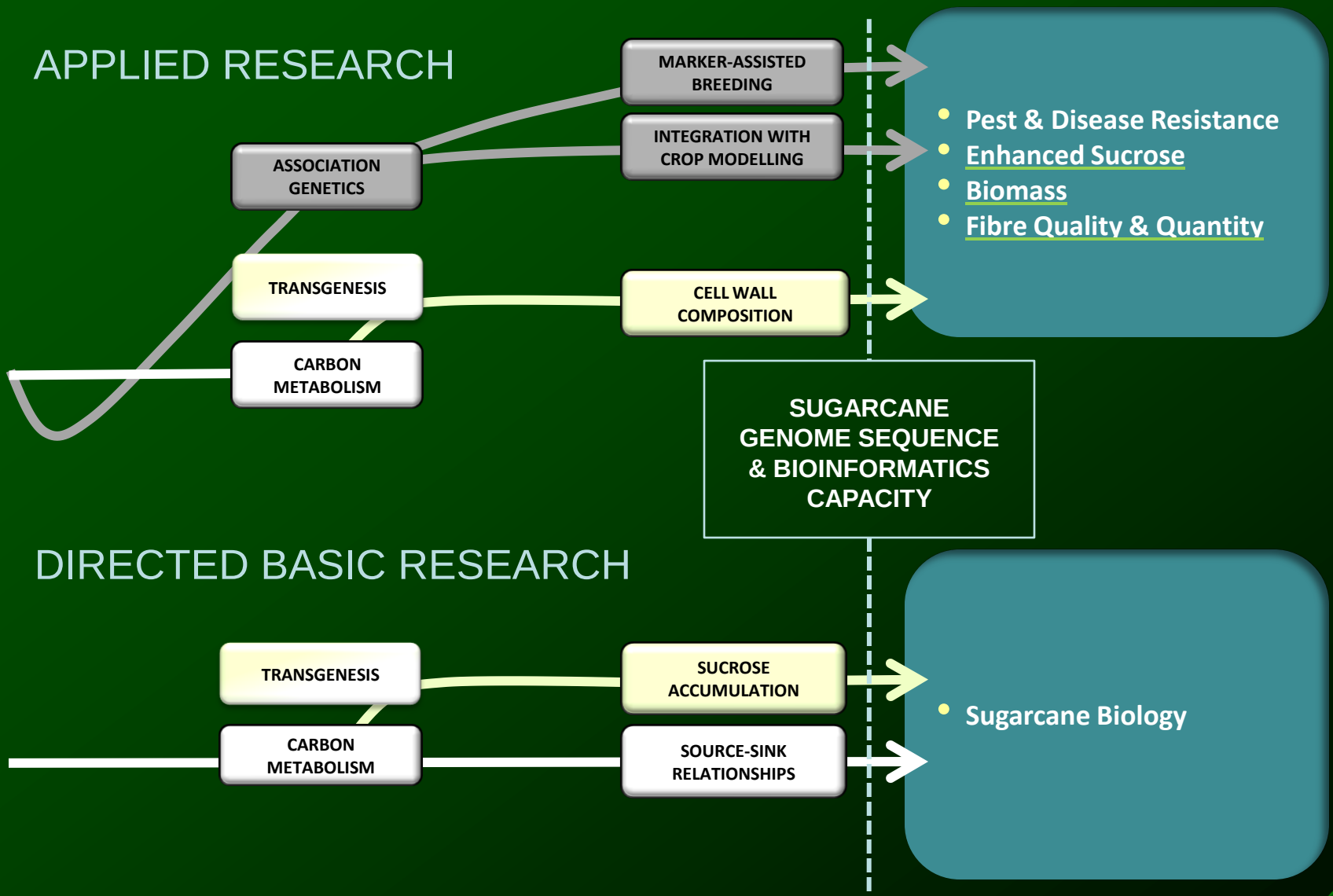
- ANALYSIS OF PHOTOSYTHESIS
- GENE EXPRESSION PROFILING
  - 3'-UTR Pyrosequencing (Eveland *et al*., 2008)
- METABOLITE PROFILING
- ENZYME ACTIVITY DETERMINATION

SUGARCANE BIOLOGY (SOURCE-SINK RELATIONSHIPS) : CURRENT RESEARCH

Changes in the Leaf Associated with Internode Maturation (increased Suc : (Glc + Fru Ratio)



CHALLENGES : GENOMICS & FUNCTIONAL GENOMICS



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Alistair McCormick
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