

Functional Genomic Approaches to Plant Stress Resistance

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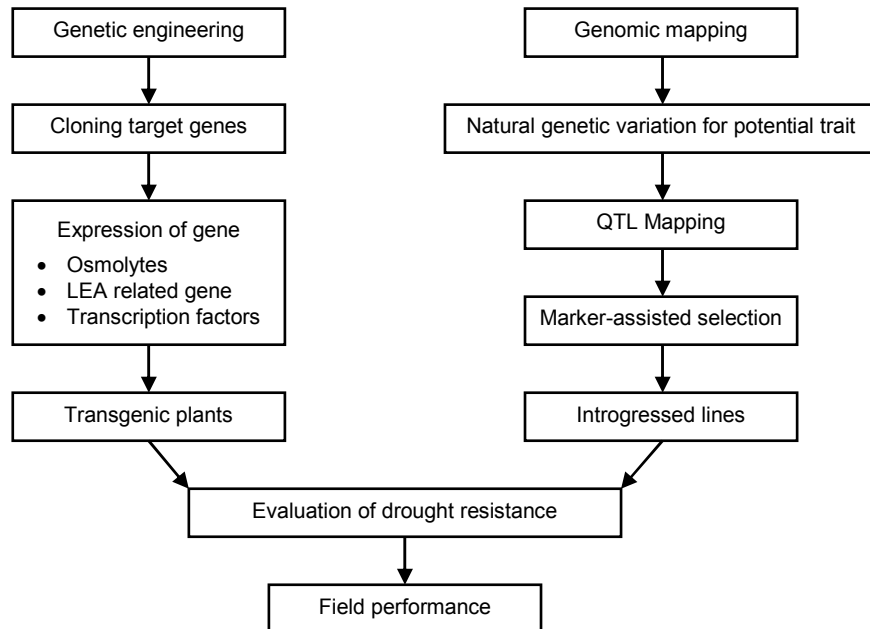
Literature Review

1. Molecular Dissection of Drought Resistance in Crop Plants : from Traits to Genes (by Henry T. Nguyen)
2. Genomic approaches to plant stress tolerance. **Current opinion in Plant Biology** 2000, 3:117-124.
3. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. **The Plant Journal** (2002) 31(3):279-292.
4. Regulatory network of gene expression in the drought and cold stress respons. **Current Opinion in Plant Biology** 2003, 6:410-417.
5. Abiotic Stress and plant genome Evolution. Search for New Models. **Plant Physiology** May 2005, vol. 138 pp. 127-130

Introduction

1. Abiotic stresses, such as drought, salinity, extreme temperature, chemical toxicity and oxidative stress are serious threat to agriculture and the natural status of the environment
2. Study of abiotic stresses should be given high research priority in plant biotechnology
3. Molecular control mechanisms for abiotic stress tolerance are based on the *activation dan regulation* of spesific stress-related genes
4. Molecular mapping and genomics approaches offer new opportunities and strategies to dissect major genes and quantitative trait loci (QTL) underlying abiotic (drought) tolerance.
5. New molecular tools are available that can be integrated with conventional breeding and physiology to accelerate a basic understanding of drought tolerance in plants and the development of drought tolerance crops.

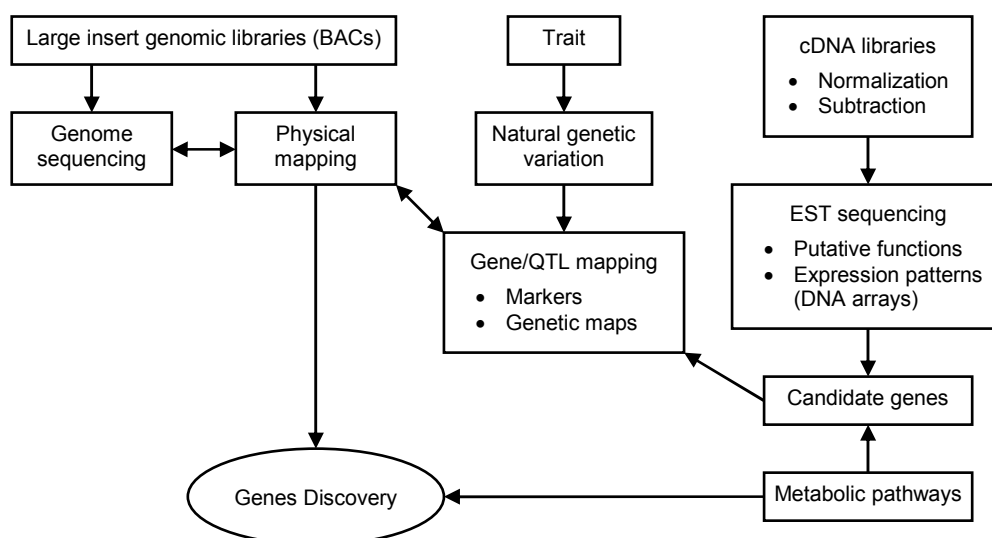
Strategies for Improving Drought Resistance



What Next

1. Past efforts to improve plant tolerance to drought, high salinity and low-temperature through breeding and genetic engineering have had limited success owing to the genetic complexity of stress responses.
2. Progress is now anticipated through comparative genomics studies of an evolutionarily diverse set of model organisms, and through the use of techniques such as high-throughput analysis of expressed sequence tags, large-scale parallel analysis of gene expression, targeted or random mutagenesis, and gain-of-function or mutant complementation

From Traits to Genes



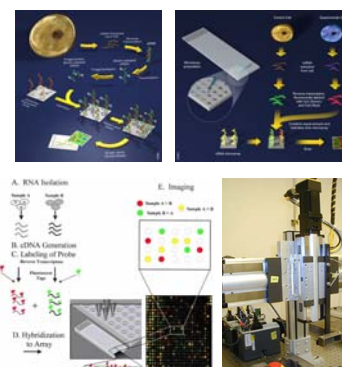
Advances in molecular marker technology and the development of integrated genetic and physical maps make gene discovery in QTL possible

Functional Genomic and Candidate Gene Approaches

1. EST (Expressed Sequence Tags) and Microarray Technology
2. Candidate Gene Analysis

1. EST (Expressed Sequence Tags) and Microarray Technology

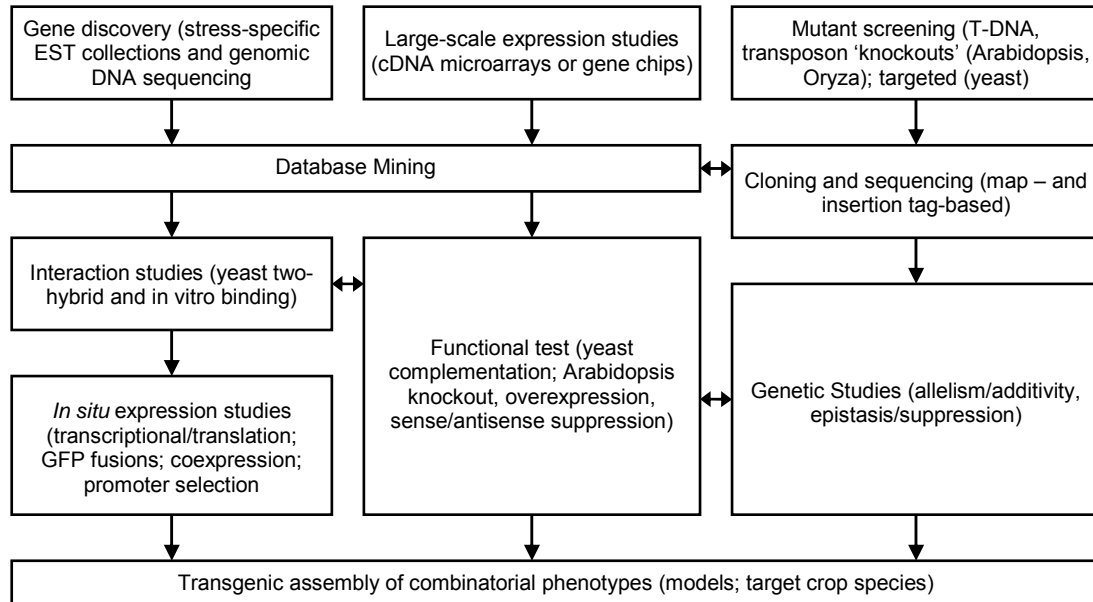
- Microarray technology allows one to monitor the expression of thousands of genes systematically in a single hybridisation using small quantities of the RNA sample.
- Requiring relatively small amounts of sources tissues
- Compared to oligo-based DNA chips, microarrays are quicker and less expensive to make, and more adaptable regarding the nucleic acid source that can be arrayed
- This technology should be extremely useful in determining the genetic foundations of complex traits like abiotic stress tolerance when coupled with drought-regulated expressed sequenced tags (EST)
- Development of unique drought related ESTs are needed in most crops to allow the utilization of microarray technology in abiotic stress tolerance gene discovery



2. Candidate Gene Analysis

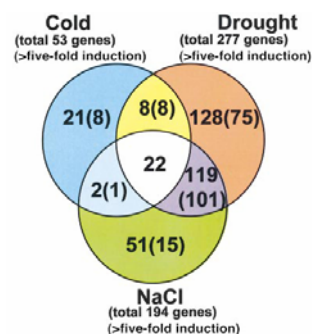
1. Candidate gene analysis starts with selection of some target genes based on biological pathway or genome location relative to a known QTL identified for the target trait
2. A drought-related EST database, microarray analysis, and the mutagenesis approach will yield a large number of valuable candidates for verification of their association with the drought tolerance traits.
3. Alternatively, searches can be conducted for orthologs in existing literature and databases for information on drought and related abiotic stress genes.
4. After genetic mapping is accomplished, ESTs that map very near the trait QTLs can be targeted for further candidate gene analysis.
5. This fine mapping of the genes that generate a QTL will only be possible in NIL populations, in which a single QTL provides all of the population variation for drought tolerance.
6. Since most of the ESTs can be located on the physical map, one will be able to target a subset of candidate genes for co-segregation analysis in different populations.
7. If several candidate genes with different functions in different QTL locations could be verified, a model can be hypothesized regarding how drought tolerance is manifested.
8. With the integration of QTL mapping, comparative mapping information, growing EST databases, expression (including microarray) results, and the identification of more and more genes in the future, the candidate gene approach will become an important and powerful tool to uncover the mystery behind the expression of quantitative traits.
9. Finally, with the development of high quality physical map and high throughput genomic sequencing technology, a combination of cDNA capture and one-pass sequencing of several BAC contigs that comprise QTLs will be a powerful strategy in search for candidate genes.

Functional Genomics Strategy from Gene Discovery to Evaluation of Stress Tolerance Phenotypes



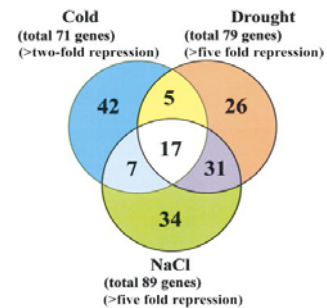
Functional Genomic using cDNA Microarray Technology

The 7000 Arabidopsis genes of full length cDNA microarray to identify new drought-, cold- or high-salinity-inducible genes:

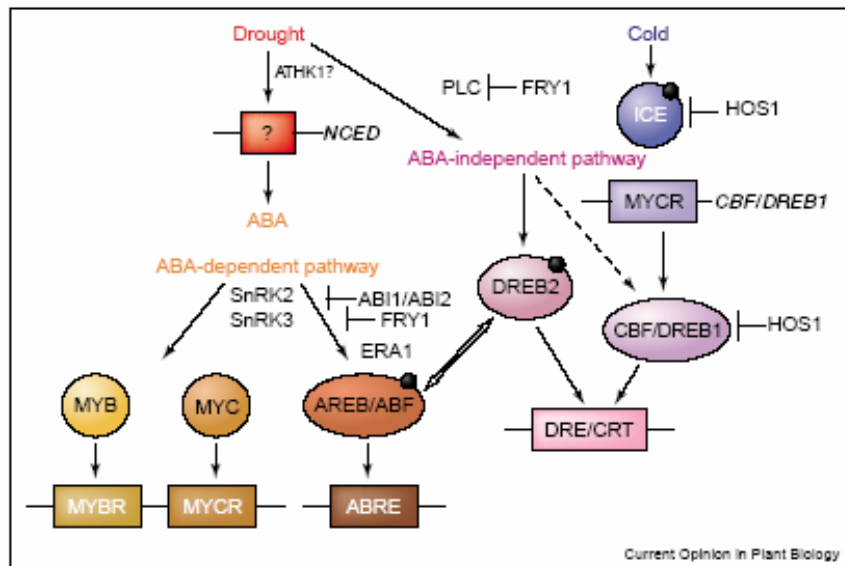


Classification of the drought, cold or high-salinity stressinducible genes identified on the basis of microarray analyses. In total, 277 drought-inducible, 53 cold-inducible and 194 high-salinity stress-inducible genes were identified by cDNA microarray analysis. The drought, cold or high-salinity stress-inducible genes identified were grouped into the following seven groups: (1) highly cold-stress-inducible; (2) highly drought-stress-inducible; (3) highly high-salinity-stressinducible; (4) drought, cold and high-salinity stress-inducible; (5) genes that were highly induced by drought and high-salinity stress; (6) genes that were highly induced by drought and cold stress; (7) genes that were highly induced by cold and high-salinity stress. The number of genes whose expression ratio is more than five fold for each stress treatment and less than five fold for the other stress treatments is indicated. Numbers in parentheses represent the number of genes whose expression ratio is more than five fold for each stress treatment and less than threefold for the other stress treatments.

Classification of the drought, cold or high-salinity stress downregulated genes identified on the basis of microarray analyses. The drought, cold or high-salinity stress-downregulated genes were grouped in the following seven groups: (1) highly cold-stress-downregulated genes; (2) highly drought-stress-downregulated genes; (3) highly high-salinity stress-downregulated genes; (4) drought, cold and high-salinity-stress-downregulated genes; (5) genes that were highly downregulated by drought and high-salinity stress; (6) genes that were highly down regulated by drought and cold stress; and (7) genes that were highly downregulated by cold and high-salinity stress.



Regulate Gene Expression in Response to Drought and Cold Stress: Identification of Cis-acting Elements and Their DNA-binding Proteins in ABA-independent Pathways



Regulatory network of gene expression in response to drought and cold stresses: specificity and crosstalk of gene networks. Cis-acting elements that are involved in stress-responsive transcription are shown in boxes. Transcription factors that control stress-inducible gene expression are shown in circles or ovals. The open double-headed arrow suggests crosstalk between DREB2 and AREB/ABF that is based on DRE/CRT's acting as a coupling element for ABRE [55]. ERA1, ENHANCED RESPONSE TO ABA1; MYBR, MYB recognition site; MYCR, MYC recognition site.

Conclusions

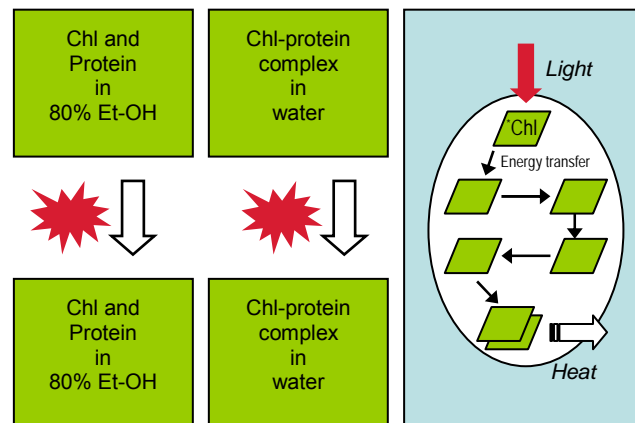
- Molecular and genomic analyses have revealed many genes that are induced by abiotic stress and their functions in stress-responsive gene expression and stress tolerance.
- Full-length cDNA microarray analysis is a powerful tool for the identification of stress-inducible genes.
- In future, a combination of molecular, genomic and genetic analysis will be used to elucidate the complex systems that regulate the responses of gene expressions to abiotic stresses.

Photosynthesis-related Stress on Plants and Their Resistance to It

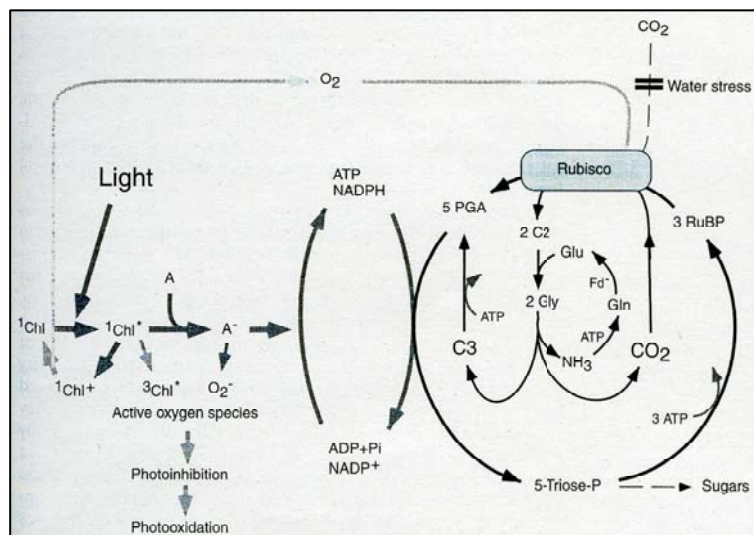
Masaaki Takahashi

Laboratory of Plant Molecular Biology, Department of Applied Life Sciences
Graduate School of Life and Environmental Sciences, Osaka Prefecture University

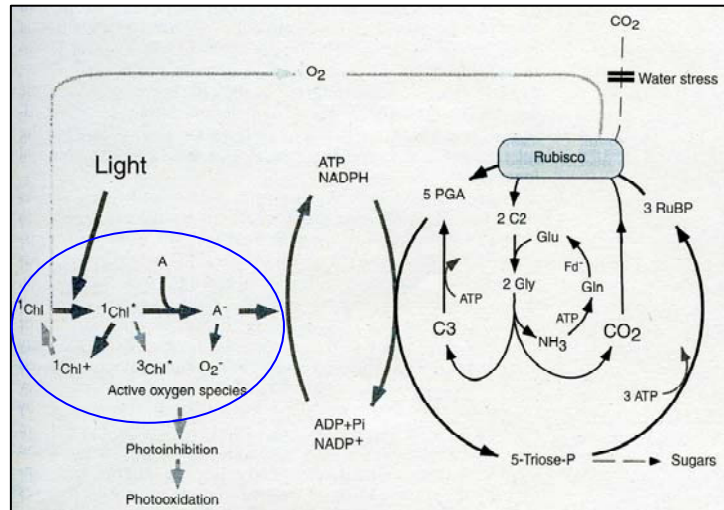
Chlorophyll Itself was Gained as a Light Guard



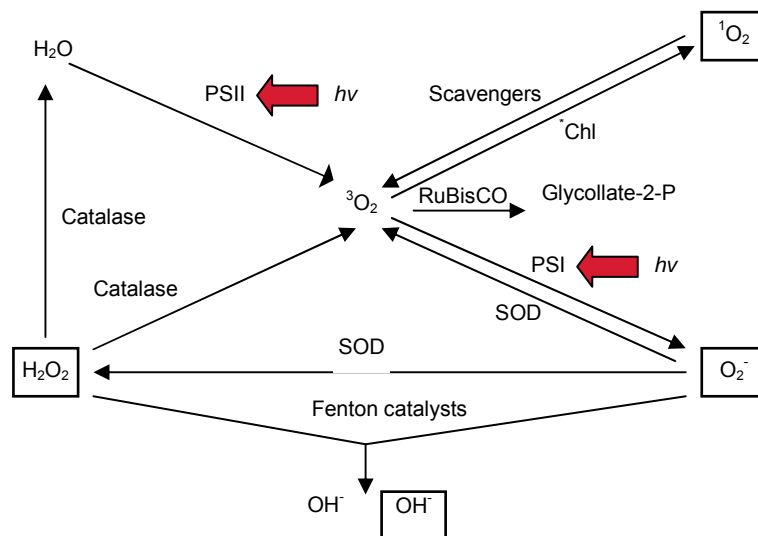
Solar Energy is Used to Drive Creative or Non-creative Reactions in Oxygenic Photosynthetic Organisms



Reactive Oxygen Species (ROS); Unavoidable Gifts from Oxygenic Photosynthetic World

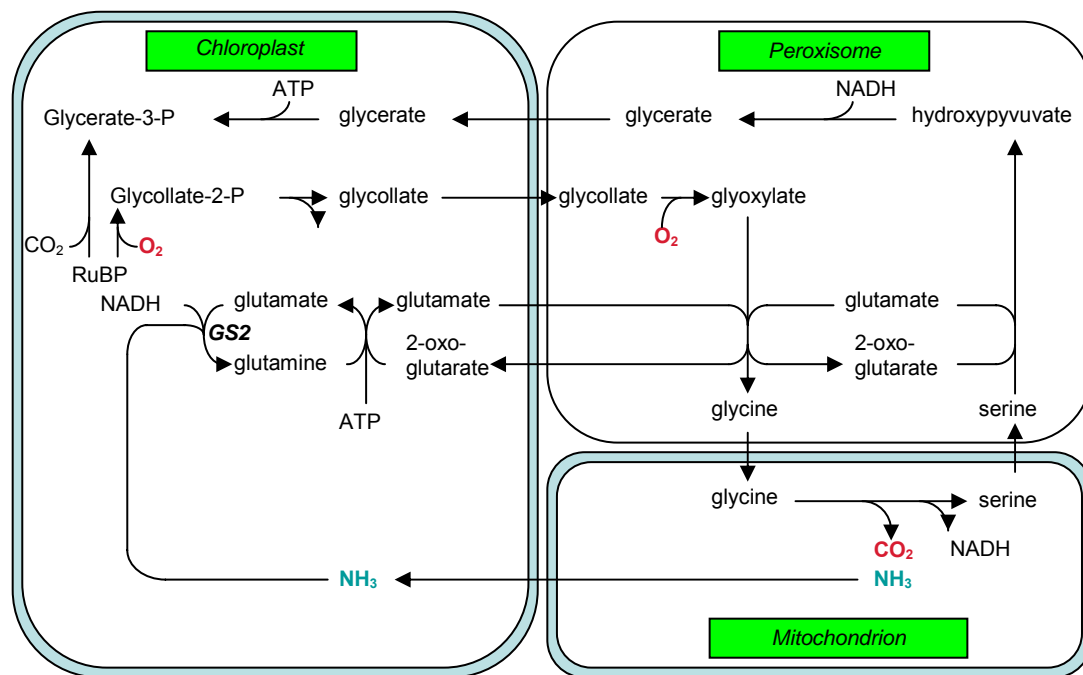


Oxygen Metabolism in the Chloroplast

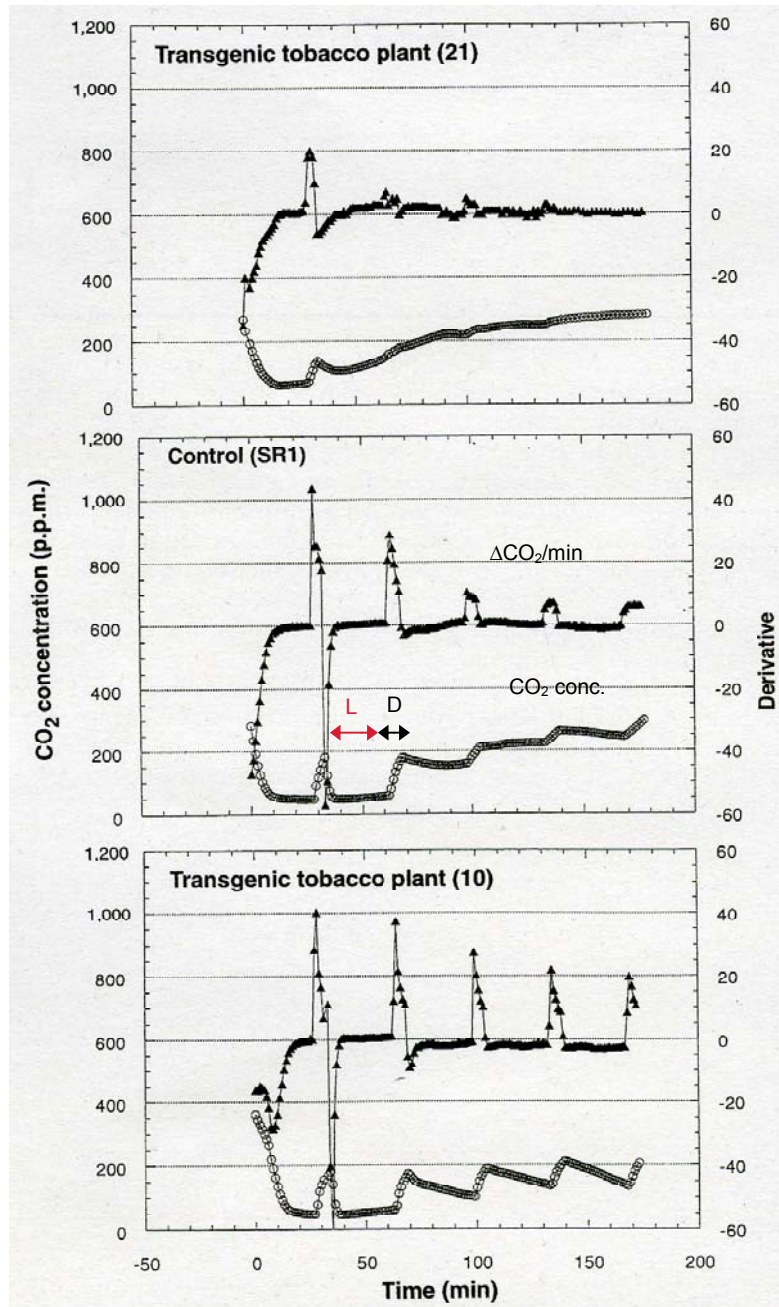


Photorespiration Protects C3 Plants from Photooxidation Akiko Kozaki and Go Takeba *Nature* 384: 557-560 (1996)

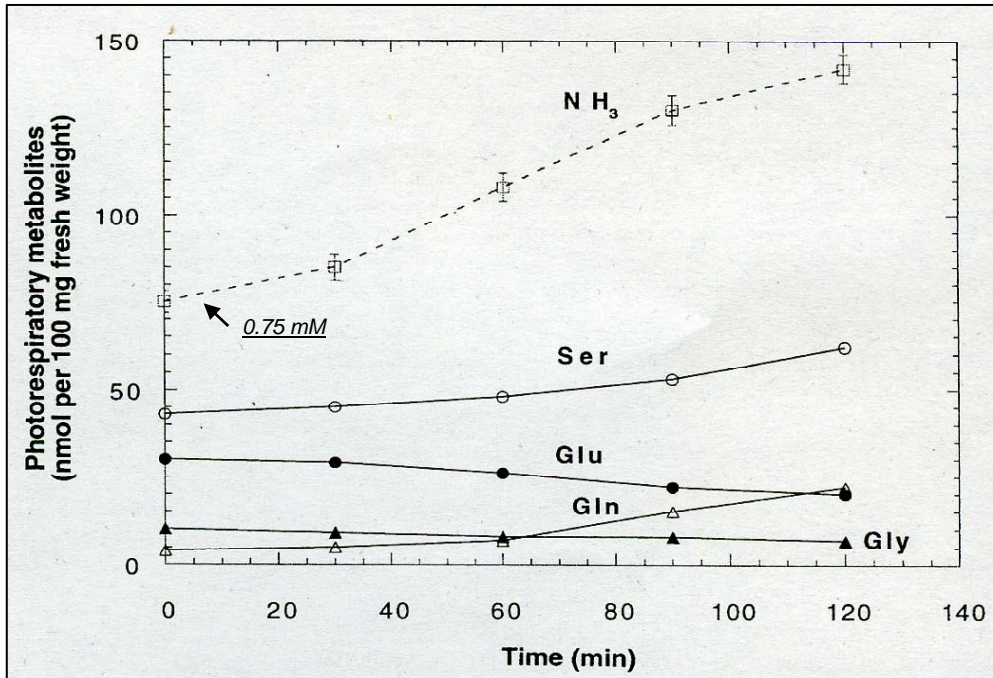
Photorespiratory Pathway



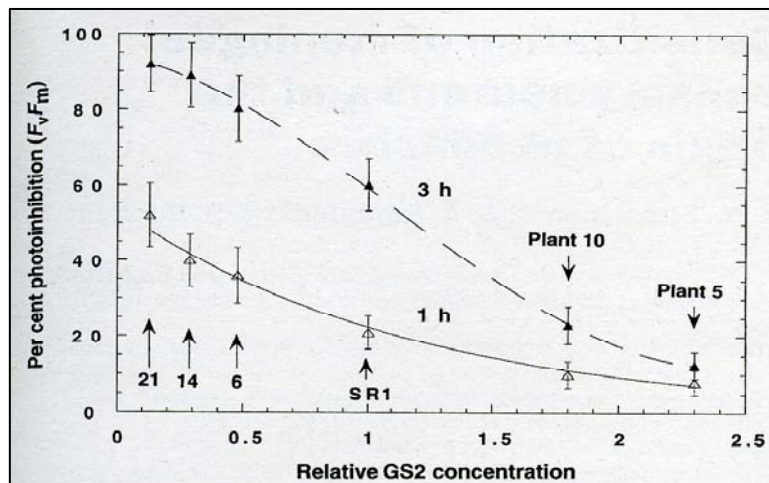
Photorespiration (CO_2 Burst) Highly Depends on Glutamine Synthetase (GS2) Activity



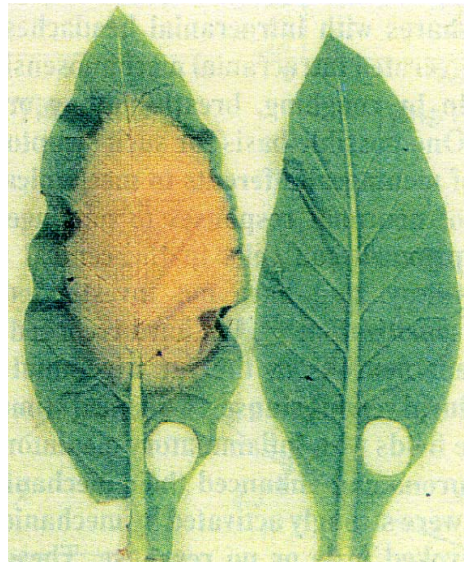
NH₃ Accumulates in Chloroplasts when CO₂ Flow is Shut Down



GS2 Transgenic Plant is Not Photoinhibited



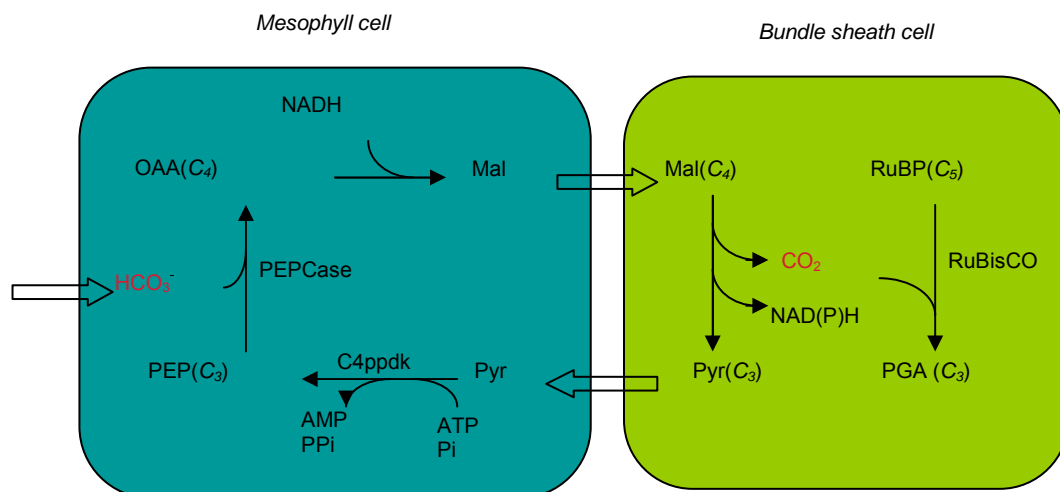
GS2 Transgenic Plant is Not Photoinhibited



Control

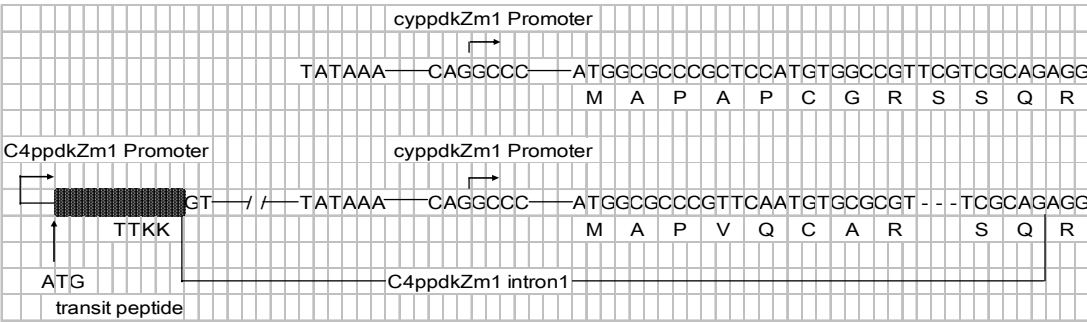
GS2 transgenic

C4 Photosynthesis was Evolved ~70 Million Years Ago to Cope with Recent Climate Changes by Dissipating Solar Energy Once Absorbed

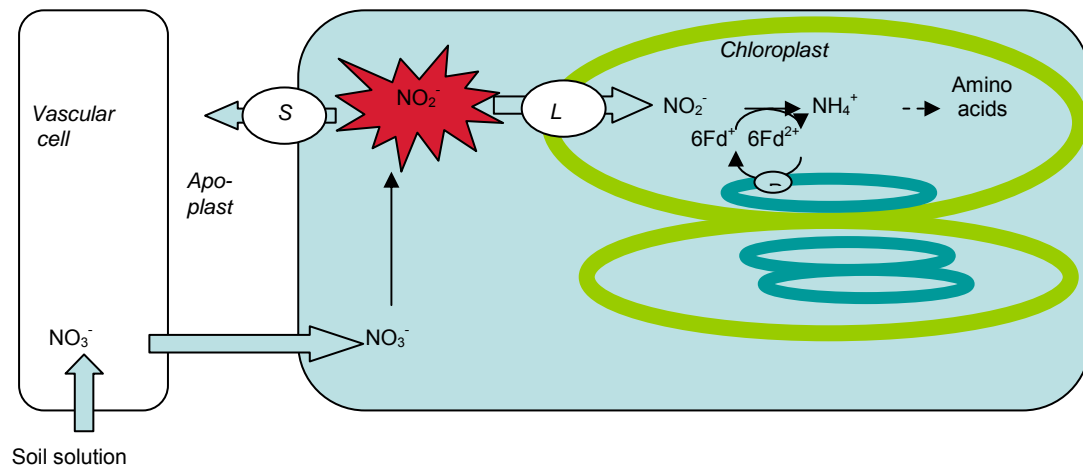


Recent Evolution of Chloroplast-type Isoform from Cytosolic Pyruvate
Pi Dikinase J. Sheen *Plant Cell* 3:225-245 (1991)

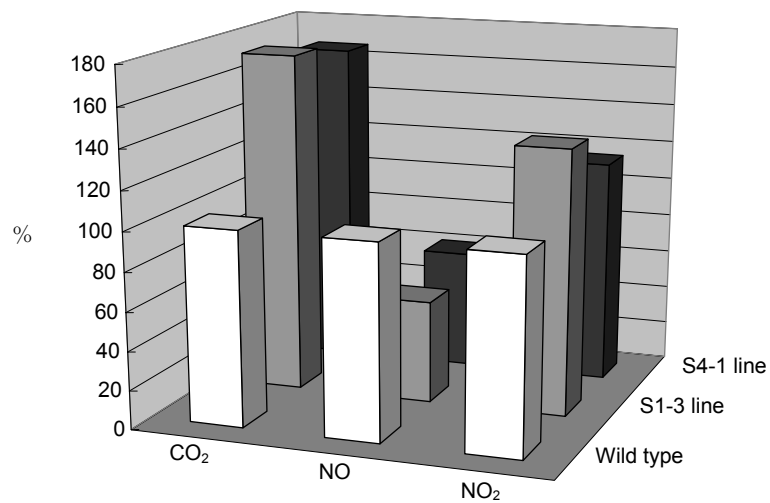
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S	E	G	N	K	N	M	K	E	L	L	G	G	K	G	A	N	L	A	E
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Q	Y	Q	D	A	G	C	A	L	P	A	G	L	W	A	E	I	V	D	G
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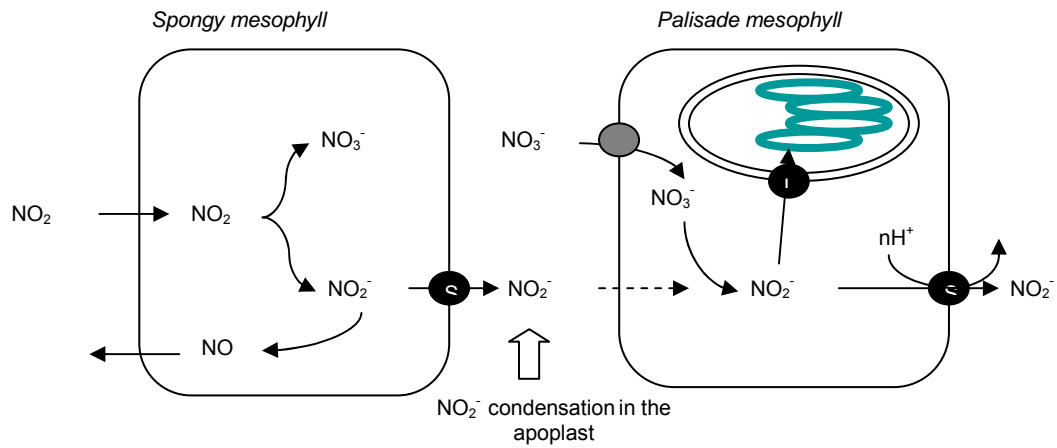
Nitrate Assimilation by Plant – The Role of the Nitrite Transporters (CsNitr1-L&-S) –



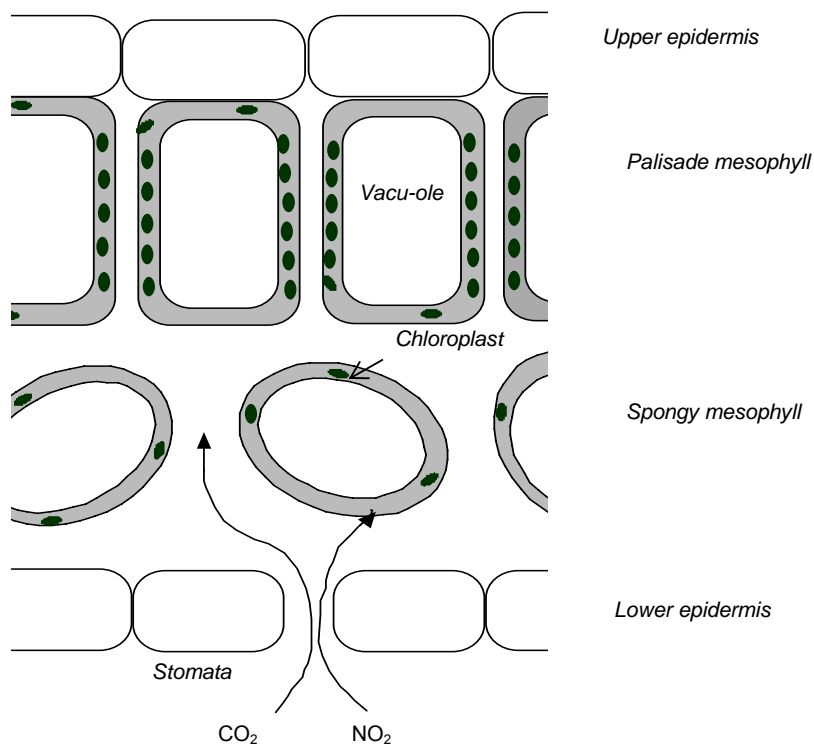
Uptake of NO_2 , NO , and CO_2 by the Nitrite Transporter (CsNitr1-S) Transgenic *Arabidopsis*



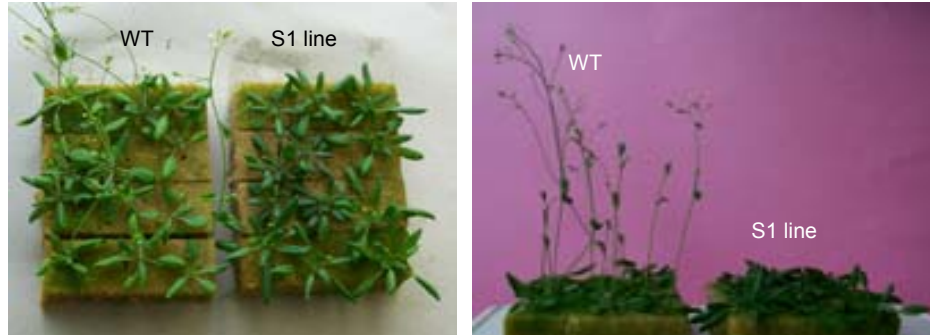
NO₂ Assimilation by the Nitrite Transporter (CsNitr1-S) Transgenic Plant



Path for the Atmospheric CO₂ and NO₂ to Plant Cells



**Phenotypic Difference of Nitrite Transporter (*CsNitr1-S*)
Transgenic *Arabidopsis* from Its Control (*Col-0*)**



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