Genetic Engineering for Enhancing Abiotic Stress Tolerance : Water Use Efficiency and Nitrogen Use Efficiency

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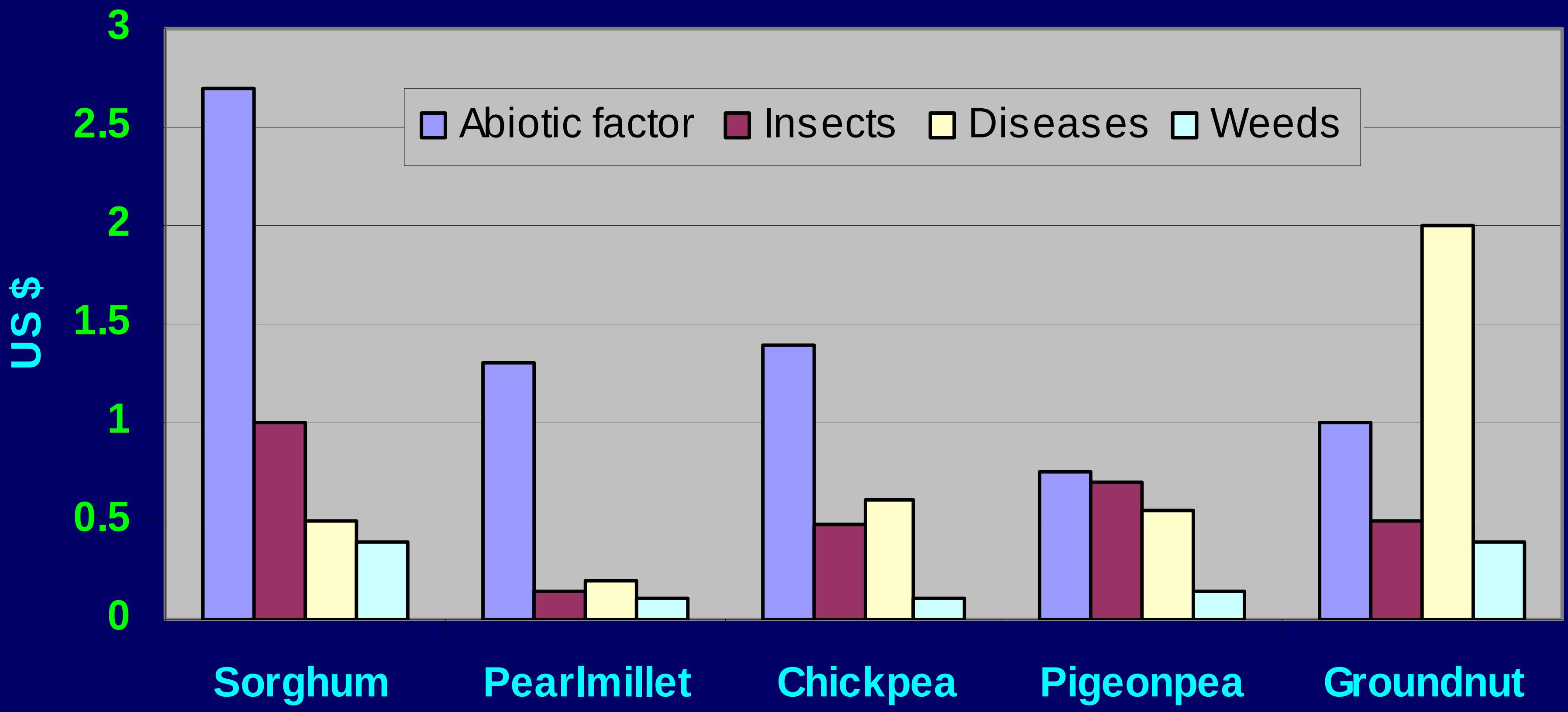
Which Trait(s) after Bt or Herbicide Resistance ?

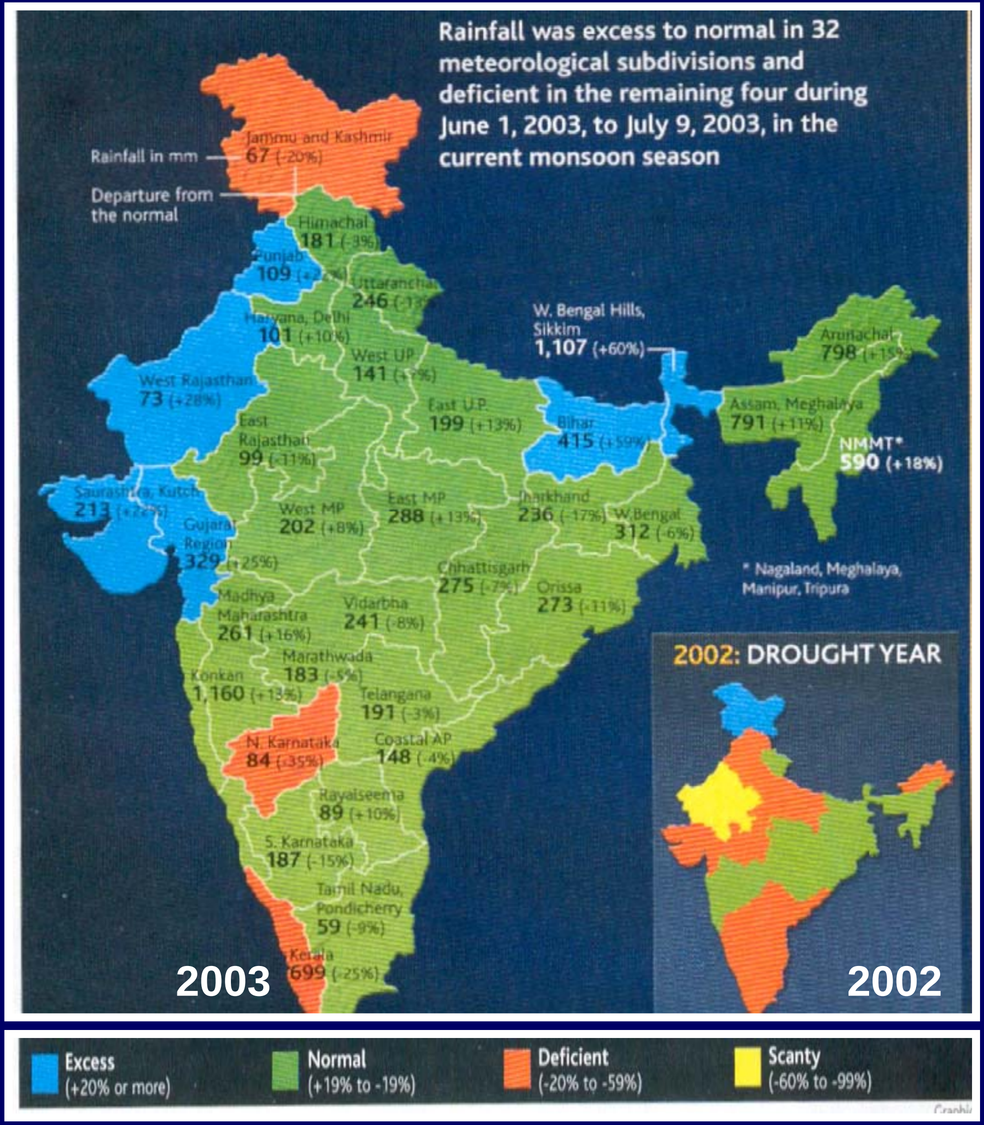
- Water Use Efficiency/Abiotic Stress Tolerance
- Nutrient Use Efficiency
- Nutritional Quality/ Edible Vaccines
- Disease Resistance: Virus resistance
- Yield and Yield Components

Loss due to Abiotic Factors, Insects, Diseases and Weeds



Source: ICRISAT, India 1992

Indian Agriculture is Dependent on Monsoon



Rainfall was excess to normal in 32 meteorological subdivisions and deficient in the remaining four during June 1, 2003, to July 9, 2003, in the current monsoon season

* Nagaland, Meghalaya, Manipur, Tripura

Total food grain production in India

Year	Production (mt)
2001	209
2002	185

Source : Economic Review (2003)

BUSINESS TIMES

The Times of India, New Delhi, Thursday, November 7, 2002

e barking robot

uard dog robot Banryu, made
y Sanyo and robotics venture
tmsuk, can transmit video
images to a mobile phone,
detect fire and bark
against dubious people

"Capital inadequacy and a high level of NPAs led the century-old private bank into a severe financial crisis forcing the Centre to put it under moratorium."

JR Prabhu, chairman, Nedungadi Ban

Mustard output to fall

Production of mustard oilseeds may fall this year due to drought in Rajasthan, which accounts for 65 per cent of rabi output, leading to larger edible oil imports

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Mustard output to fall

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November 7, 2002

Biotechnological Strategies to increase input use efficiency

- Marker assisted breeding
- Transgenic development
- Genomics, proteomics
- TILLING/EcoTILLING
- Search for new genes

GENES FOR ABIOTIC STRESS TOLERANCE

International Effort

Gene/QTL	Tolerance
HARDY **gene**	**Drought**
Sub1 (QTL)	**Submergence tolerance**
OsDREB1A	**Drought, high-salt and low-temperature stresses**
otsA and otsB **- trehalose biosynthesis**	**Drought and/or salinity**
CBF1	**Salinity, drought and chilling**
HSFs	**Heat stress**
SNAC1	**Drought resistance and salt tolerance**
OsCOIN	**Chilling, salt and drought, and enhanced proline levels**
ABF3	**Drought and cold**

New Ways to Protect Drought-Stricken Plants

Anne Simon Moffat. Science 296:1226-1229, May 17 2002.

With drought an ever-present threat, researchers are identifying genes that can help plants tolerate arid conditions in hopes of using them to produce hardier crops.

Tomato plants carrying a foreign gene that protects their cells from salt-induced dehydration thrive in a 200-mM salt solution, whereas unaltered plants wither.

SOURCE: ED BLUMWALD/UNIVERSITY OF CALIFORNIA, DAVIS

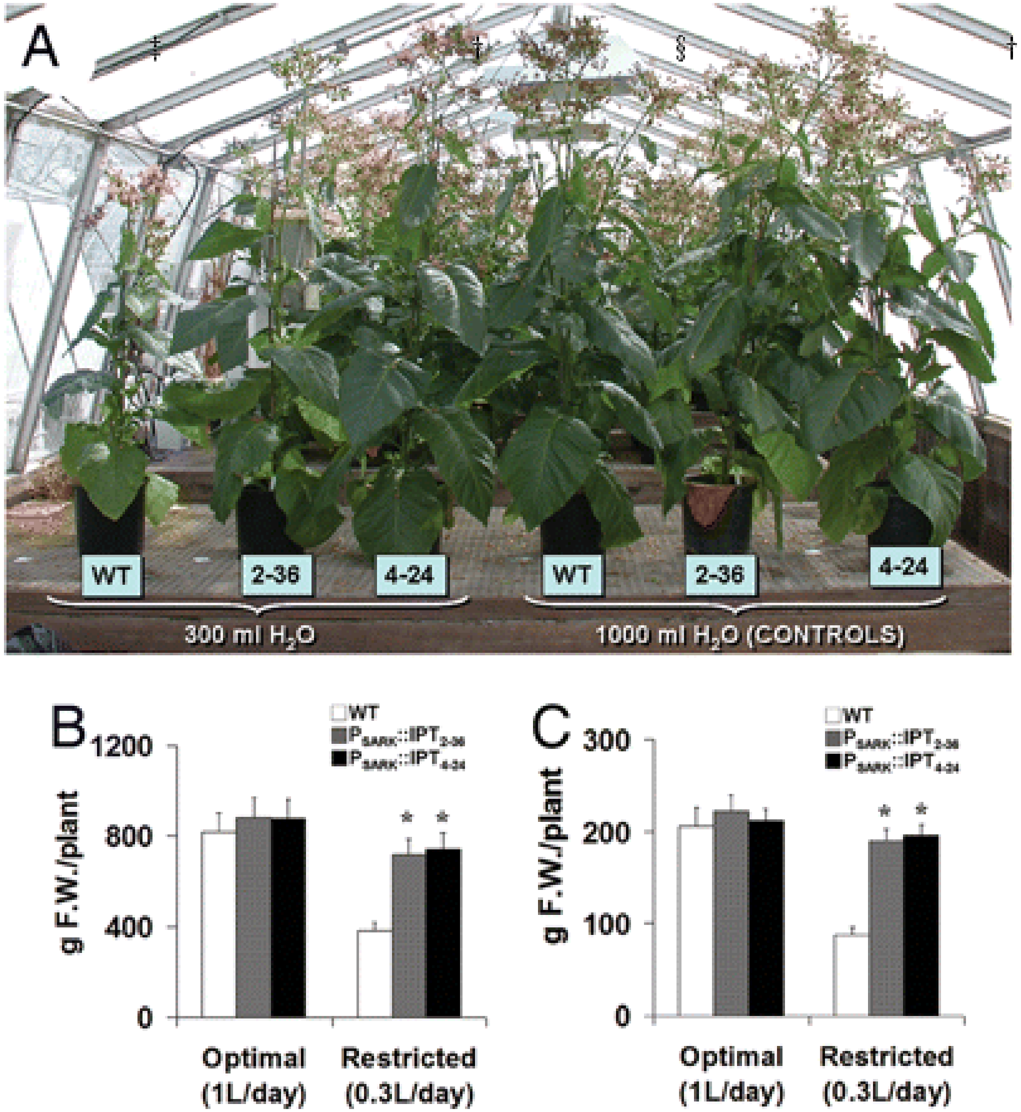
Transgenic Tomato

Wild Type

†

Delayed leaf senescence induces extreme drought tolerance in a flowering plant

Rosa M. Rivero*, Mikiko Kojima , Amira Gepstein , Hitoshi Sakakibara , Ron Mittler , Shimon Gepstein , Eduardo Blumwald*



Comparison between WT and transgenic $P_{SARK}::IPT_{2-36}$ and $P_{SARK}::IPT_{4-24}$ tobacco plants at optimal (1 liter/day) or restricted (0.3 liter/day) watering regimes. (*A*) Plants after 4 months of treatments. (*B*) Plant fresh weight at the end of the experiment. (*C*) Seed fresh weight at the end of the experiment. Asterisks indicate significant differences ($P < 0.001$) between the transgenic lines and WT. Values are the mean ± SE ($n = 24$).

Salt Tolerant Canola

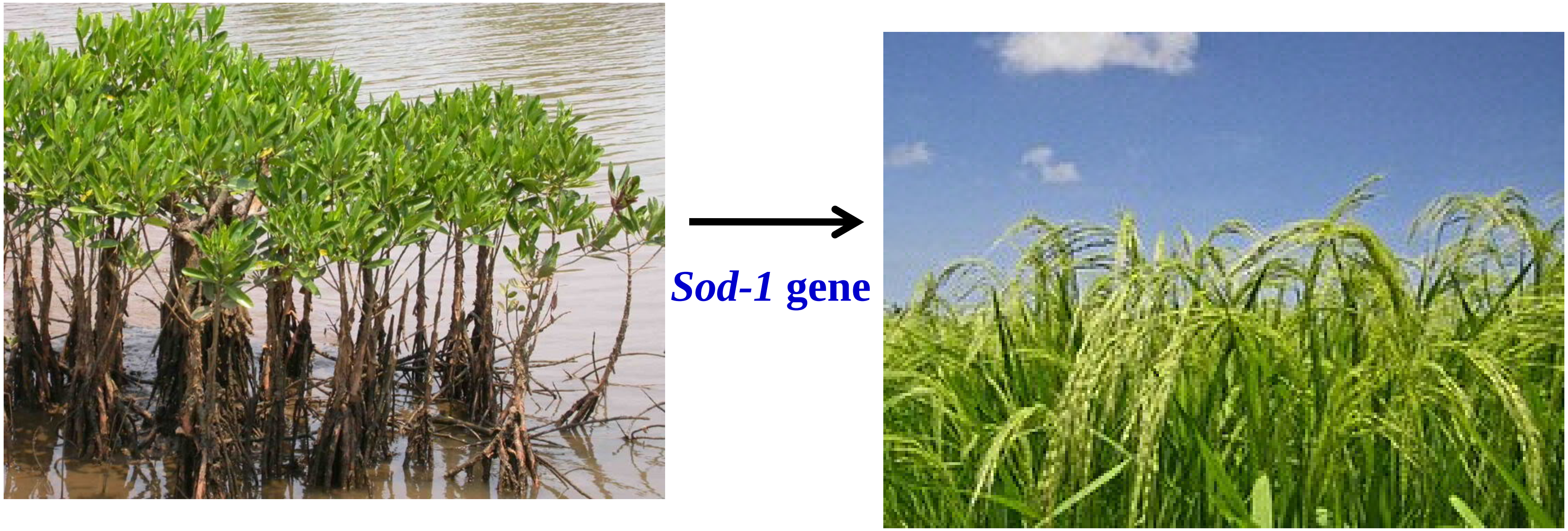
Source: Ed Blumwald, USA

Abiotic Stress Tolerance: Indian Efforts

BIOPROSPECTING OF GENES AND ALLELE MINING

- Prospecting novel genes, promoters and alleles for economically important traits using indigenous bio-resources
- Transfer the validated genes and alleles to recipient species cutting across biological barriers

Salt Tolerant Rice



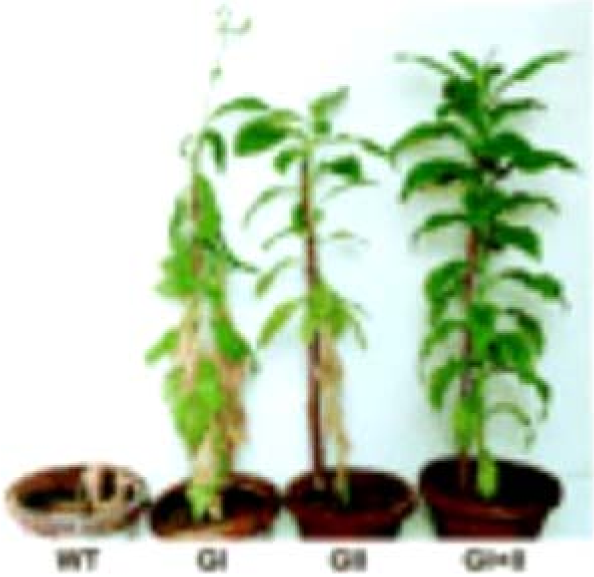
***Sod-1* gene**

Tolerates 100 mM NaCl

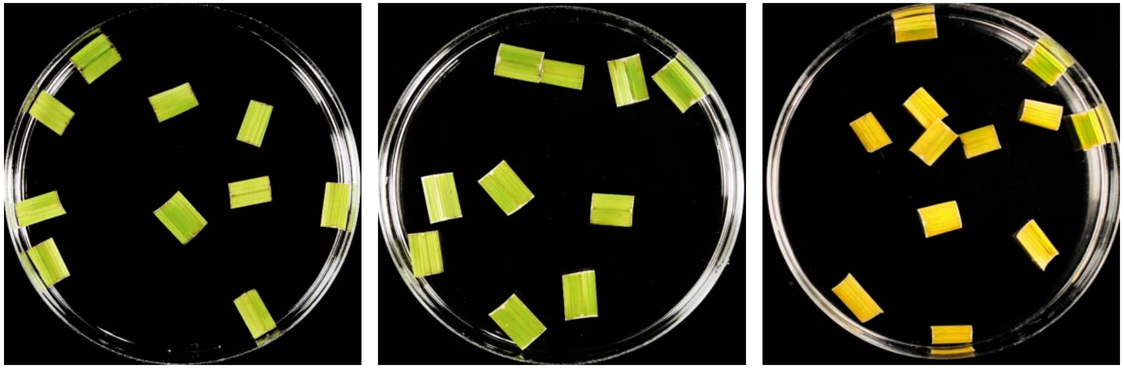
Superoxide dismutase (*Sod-1*) gene from Mangrove plant (*Avicennia marina*)

Source: Ajay Parida, MSSRF

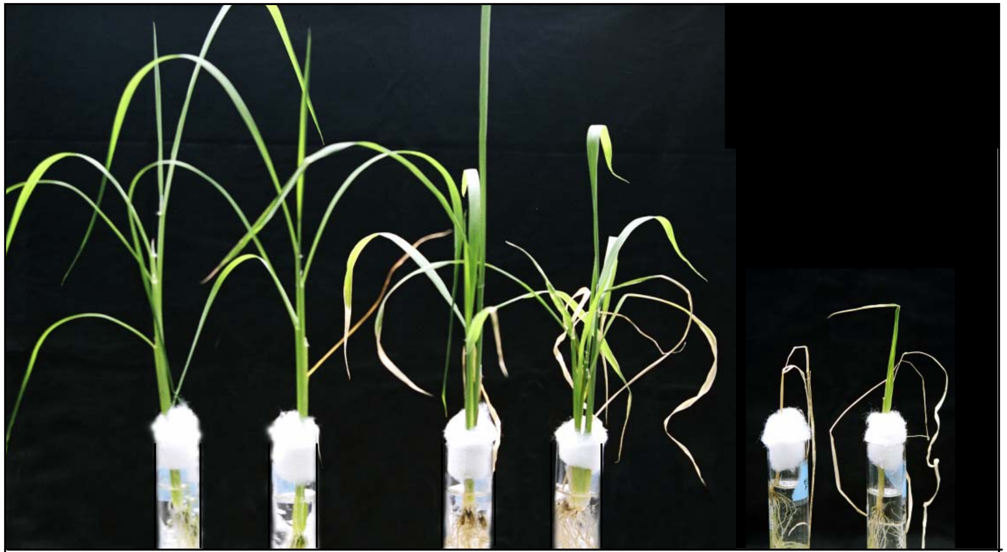
Genetic transformation of *Indica* Rice with *Glyoxalase* I and *Glyoxalase* II for Enhanced Salinity Tolerance



Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance
S. L. Singla-Pareek, M. K. Reddy and S. K. Sopory *
PNAS **2003** vol. 100 no. 25, **14672-14677**



PB1-glyI-1 **PB1-glyI-2** **Non-transgenic PB1**

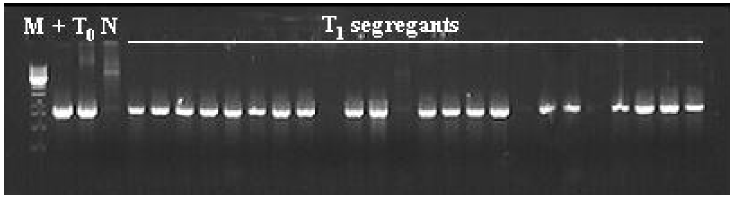


***glyII* & non-trans**
un stressed

PB- *gly*II-3
100 mM NaCl

non-trans
100 mM NaCl

Biolistic transformation of IR64 and PB1 using *glyI* and *glyII*



Inheritance of *glyII* in T$_1$ transformants

Source: Sudhir Sopory, ICGEB

GENES FOR ABIOTIC STRESS TOLERANCE CLONED AT NRCPB (ICAR)

S. No.	Gene	Source	Accession #
1.	Late embryogenesis abundant protein 1a (*BjLEA1a*)	*Brassica juncea*	DQ166625
2	Late embryogenesis abundant protein 1a (*BcLEA1a*)	*Brassica carinata*	DQ166626
3	Late embryogenesis abundant protein 1b (*BcLEA1b*)	*Brassica carinata*	DQ166627
4	Late embryogenesis abundant protein 1a (*BnLEA1a*)	*Brassica napus*	DQ178982
5	Late embryogenesis abundant protein 1b (*BnLEA1b*)	*Brassica napus*	DQ178983
6	Late embryogenesis abundant protein (*BcLEA1*)	*Brassica carinata*	AY572959
7	Late embryogenesis abundant protein (*BnLEA1*)	*Brassica napus*	AY572958
8	Transcription factor, C-repeat DNA binding protein (*TaCBF2*)	*Triticum aestivum*	AY572831
9	Transcription factor, C-repeat DNA binding protein (*TaCBF3*)	*Triticum aestivum*	AY428036
10	AP2 transcription factor (*SHN1*)	*Brassica carinata*	DQ166624
11	C2H2 zinc finger 2 (*ZF2*) mRNA, partial	*Brassica carinata*	DQ166622
12	AP2 transcription factor (*SHN1*)	*Brassica juncea*	DQ166623
13	C2H2 zinc finger 2 (*ZF2*) mRNA, partial	*Brassica napus*	DQ178980
14	C2H2-type zinc finger protein (*ZF1*)	*Brassica carinata*	DQ166621
15	C2H2-type zinc finger protein (*ZF1*)	*Brassica napus*	DQ178981
16	LEA protein (*SiLEA1*)	*Sisymbrium irio*	AY950638
17	Protein-farnesyltransferase beta subunit (*ERA1*)	*T. aestivum cv. C306*	DQ858293
18	*TatAPX* - Thylakoid bound ascorbate peroxidase	*T. aestivum cv. C306*	

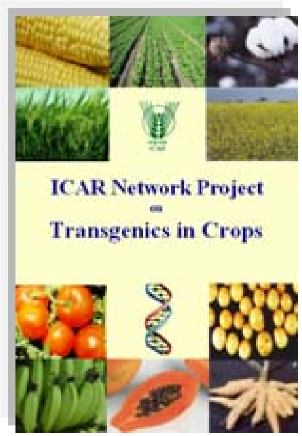
Abiotic Stress-inducible Promoters cloned at NRCPB (ICAR)

S. No.	Promoter	Source	Accession #
1	*BcLEA1* - Late embryogenesis abundant protein	*Brassica carinata*	AY804188
2	*BnLEA1* - Late embryogenesis abundant protein	*Brassica napus*	AY66378
3	*BjLEA1* - Late embryogenesis abundant protein	*Brassica juncea*	AY940036
4	*MYB* 02	Rice cv N22	EU003972
5	*MYB* 04	Rice cv N22	EU003973
6.	*Zeaxanthin epoxidase*	Rice cv N22	EU007441

Crops that are being engineered with abiotic stress resistant trait

Crop	Abiotic Stress type
Rice	Drought, Salinity
Wheat	High temp., Drought, Salinity
Sorghum	Drought
Maize	Water logging, drought
Chickpea	Drought, Cold tolerance
Pigeon pea	Salinity, Drought
Groundnut	Drought
Sugarcane	Drought, Water logging
Potato	Drought, High temperature, Salinity
Mustard	Drought, Salinity
Tomato	Drought, Salinity
Cotton	Drought, Salinity

ICAR Network on Transgenics in Crops



ICAR Network Project
on
Transgenics in Crops

ABIOTIC STRESS TOLERANCE

Coordinator: K C Bansal

TOMATO

- NRCPB
- IIVR
- IIHR

MUSTARD

- NRCPB
- NRCRM

RICE

- NRCPB
- DRR
- CRRI

Transgenics
Abiotic Stress Tolerance

WHEAT

- NRCPB

GROUNDNUT

-NRCG
-NRCPB

COTTON

-CICR
-NRCPB

Indo-US Agricultural Knowledge Initiative (AKI)
Genetic Engineering for Abiotic Stress Tolerance in Crops

Rice
Wheat
Sugarcane
Cotton

Gene/Promoter
cDNAs
Arabidopsis CBF3
Brassica ZF1
Arabidopsis AVP1
Triticum aestivum thylakoid bound *APX*
*Oryza sativa LEA*3-1/HVA1
Arabidopsis HSP101
Triticum aestivum SSS
Triticum aestivum ADPgase
Arabidopsis NHX1
Arabidopsis AVP1

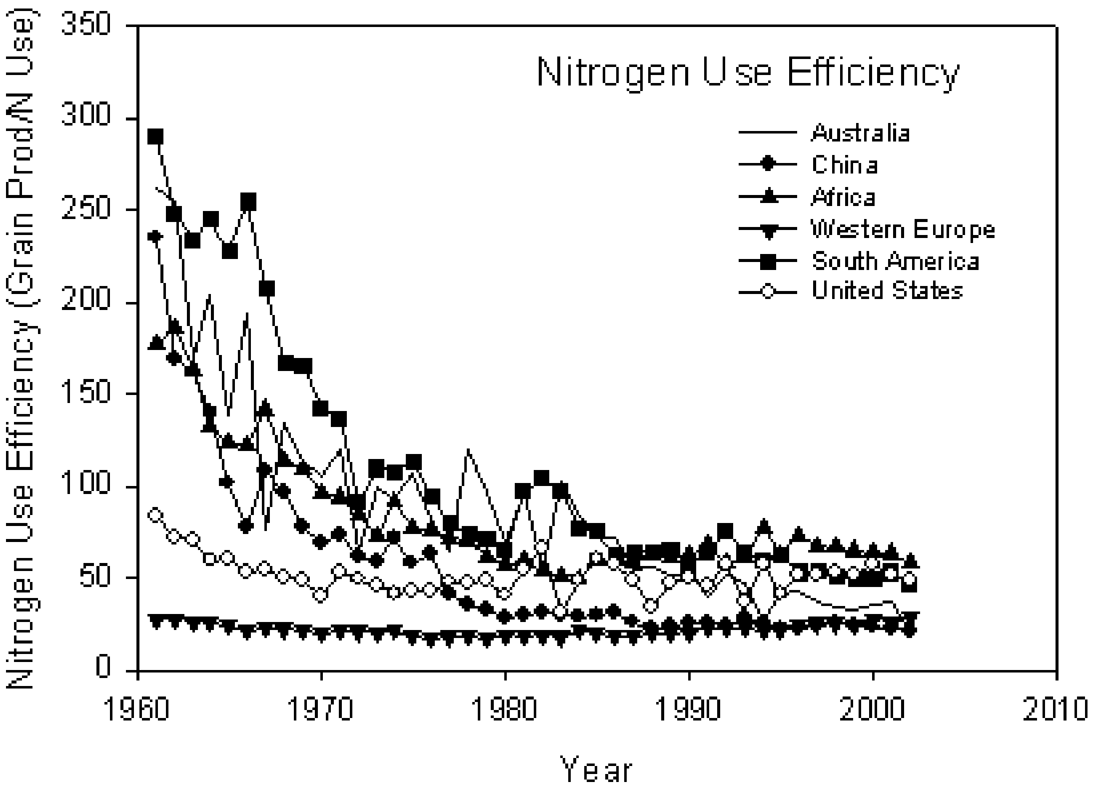
Promoters
AtRD29A, *OsMYB02*, *BcLEA1*, *TaHSP26* *TaHSP16.9*

Transgenic osmotin tomato undergoing field testing at IARI fields (2005-06)

Transgenic Crops undergoing Field Trials at the International Level

Transgenic Crop	Gene	Stress	Location	Organization
Soybean	NF-YB1	Drought	Argentina	Monsanto
Wheat	DREB	Drought	Mexico	CIMMYT
Rice	Stz	Drought & Salinity	Belgium	Crop Design
Rice	DREB1	Drought	Philippines	IRRI
Tomato	AtNHX1	Salinity	USA	Arcadia Bioscience

Nitrogen use efficiency is decreasing globally



Nitrogen use efficiency for grain production relative to N fertilizer use for 1961-2002 for selected regions in the world.

Source : FAO, 2004

Improved N Use Efficiency in Maize through Genetic Engineering

A control leaf without the nitrogen stress transgene (left) compared to a leaf with the nitrogen stress transgene (right) from plants grown in a nitrogen stress environment.

Conversion of C3 Crops to C4: A global effort

Goal

Increasing crop productivity of wheat and rice by developing improved cultivars with a potential of capturing atmospheric CO_2 with far greater efficiency with less input of water and nitrogen using the modern tools of science

THE C4 RICE CONSORTIUM

George Bowes Florida	Thomas Brutnell Cornell	James Burnell James Cook	David Dawe FAO	Gerry Edwards Washington State	John Evans ANU
Julian Hibberd Cambridge	Peter Horton Sheffield	Xinguang Zhu Shanghai	Fritz Kreuzaler Aachen	Jane Langdale Oxford	Richard Leegood Sheffield
Peter Mitchell Sheffield	Erik Murchie Nottingham	Timothy Nelson Yale	John Raven Dundee	Rowan Sage Toronto	Susanne von Caemmerer ANU
Daniel Voytas Iowa	Peter Westhoff Dusseldorf	Christoph Peterhaensel Aachen	Udo Gowik Dusseldorf	Rice Science for a Better World INTERNATIONAL RICE RESEARCH INSTITUTE	

The Main C4 Rice Roadmap

Phase I

1. Discover genes responsible for Kranz Anatomy
2. Build a molecular toolbox to generate mesophyll and bundle sheath specific expression in rice.
3. Investigate factors essential for effective compartmentalization of photosynthesis.
4. Insert and functionalize C$_4$ biochemistry factors in rice.

6 YEARS

Phase II

1. Insert and functionalize genes for Kranz anatomy in rice.
2. Evaluate the impact of Kranz anatomy on the metabolism of rice.
3. Evaluate the impact of C4 metabolism on anatomy and whole plant function in rice.
4. Combine genes for Kranz anatomy and C4 biochemistry and evaluate their impact on rice.

5 YEARS

Phase III

1. Optimize C$_4$ biochemistry and Kranz anatomy in rice.
2. Introducing C$_4$ Syndrome into locally adapted cultivars throughout Asia.

4 YEARS

1st three years of the Phase I funded by a Bill & Melinda Gates Foundation grant to IRRI and the C4 Consortium

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Plant Science 172 (2007) 1204–1209

PLANT SCIENCE

www.elsevier.com/locate/plantsci

Enhanced photosynthesis rate in genetically engineered *indica* rice expressing *pepc* gene cloned from maize

A. Bandyopadhyay [a,b,1], K. Datta [a,b], J. Zhang [c], W. Yang [d],
S. Raychaudhuri [e], M. Miyao [f], S.K. Datta [a,*]

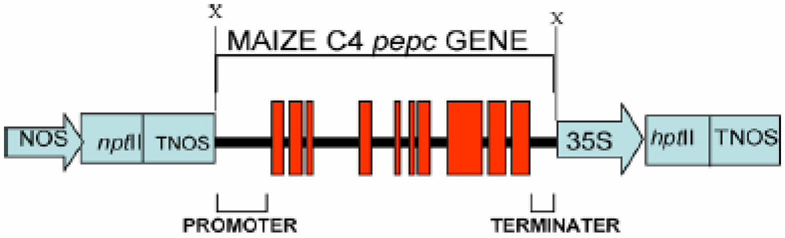
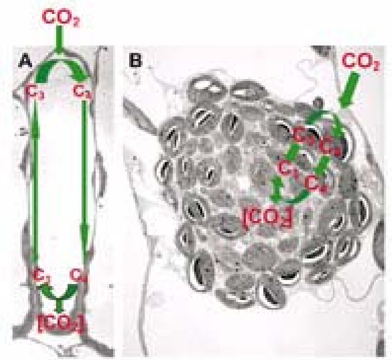


Fig. 1. Schematic diagram of the intact maize pepc gene and the selective antibiotic resistant gene hygromycin phosphotransferase (hph) used for rice transformation. The maize *pepc* gene is an 8.8 kb fragment containing all exons, introns and the promoter (from − 1212) and the terminator (2.5 kb) sequences. X = *Xba*I digestion site.

Fig. 2. Transgenic plants showing normal phenotype and good seed setting like control plants.

Towards Development of a Single Cell C4 Photosynthesis System in Rice

NAIP (ICAR)



Objectives

- Identification of new single cell C_4 photosynthesis system among the available chenopod species.
- Identification of genes involved in transition from C_3 to C_4 in single cell system and determination of cytoskeleton in single cell C_4 photosynthetic system.
- Cloning and characterization of C_4 photosynthetic genes from a single cell C_4 photosynthetic species and a C_4 plant maize/sorghum.
- Transformation of rice and tobacco and/or *Arabidopsis* with C_4 pathway genes and the functional validation of the transgenics.

Thank You