Can Synthetic Genes Lead to Synthetic Life?
Tweaking plants to manage abiotic stress

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STRESS

INTRODUCTION

BIOTIC

- Salt or Ion stress
- Drought Stress

ABIOTIC

- High Temperature
- Cold Stress
INTRODUCTION

• We all know that Agriculture is totally dependent on climate

• A variety of Abiotic Stresses cause a serious crop loss of >50% on an average, thus limiting the agricultural productivity worldwide (Wang et al., 2003)

• Water deficit (drought) has affected 64% of the global land area, flood (anoxia) 13% of the land area, salinity 6%, mineral deficiency 9%, acidic soils 15% and, cold 57% (Mittler, 2006; Cramer et al., 2011)

• World Bank projects that climate change will depress crop yields by 20% or more by the year 2050. (Tuteja et al., 2012)
Generic signaling pathway involved in plant abiotic stress responses

Wang et al. 2016
Drought resistance mechanism designed by nature

Adaptation
Ability to maintain tissue hydration

Tolerance
Ability to function while dehydrated

Escape
Ability to complete life cycle during wet period (short life cycle)
Plant abiotic stress response and intervention points for genetic engineering strategies
Information of stress responsive genes

Advancement of high throughput technique

Dinakar and Bartels, 2013

Das et al., 2015
Responsive genes from extremophiles

- Tolerance against abiotic stresses is genetically controlled in *Xerophyta viscosa* (Baker), a monocotyledonous resurrection plant (Farrant et al., 2015)

**Xerophyta viscosa**

A model African Extremophile

- Survives in extremes of dehydration and regains normal life on rehydration

![Responsive genes from extremophiles diagram](image)
Other sources of responsive genes

- **Arabidopsis thaliana** (*AtCAMTA1-6*)
- **Nicotiana tabacum** (*NtROS2a*)
- **Holomonas elongata** (*NhaD*)
- **Saccharomyces sp.** (*TPSI*)
- **E. Coli** (*otsA and otsB*)
- **Arthrobacter globiformis** (*codA*)
Winter Flounder Fish- Antifreeze protein

*Hordeum vulgare*- Hb gene

*Oryza sativa*- Cu/Zn SOD

*Vitroscilla stercoraria*- VHb gene

*Anasystis nidulans*- Fatty acid desaturase gene
Drought accounts alone for 50% of losses caused by biotic and abiotic stresses.

By 2025, 30% of crop production will be at risk due to the declining water availability (Wang et al., 2003).

Impacts of abiotic stresses on crop production:

- Drought: 64%
- Cold: 57%
- Acidic soil: 15%

Cramer et al., 2011
Drought has increased globally from 1900 to 2002

- By 2020, between 75-250 million people in Africa will be exposed to water stress due to climate change (IPCC)
- By 2025, up to 2.4 billion worldwide will be living in areas subject to periods of intense drought 50 million in areas subject to desertification (UNCCD)

Taken from www.climatecommunication.org
Drought has increased globally from 1900 to 2002,
(Adapted from Dai et al. (2004b)}
Use of genetic engineering to transform plant

Major ways to improve the stress response

1. Improving protection from stress.
   Oxidative stress is protected by SOD enzyme

2. Reducing sensitivity to stress.
   Drought tolerance, salt tolerance and chilling tolerance

Transgenic plant

- Upregulate the existing gene
- Integrate gene from other species
Ways to prevent drought

- Photosynthesis
- Hormonal regulation
- Transpiration and stomatal conductance
- Root morphology
- Osmotic adjustment

Basu et al., 2016
Strategies for the genetic engineering of drought tolerance

Umezawa et al., 2006
Drought response

Functional proteins
- Detoxification enzymes

Protection factors of macromolecules (LEA proteins, chaperones)

Key enzymes for osmolyte biosynthesis (proline, sugars)

Water channels, Transporters

Transcription factors (DREB2, AREB, MYC, MYB, bZIP, NAC HB etc.)

Protein kinases, Phosphatases, Phospholipid metabolism

Regulatory proteins

ABA biosynthesis

Proteases
Engineering drought tolerance using functional proteins

**Osmolyte metabolism:** Fructan, Trehalose, Proline, Trehalose, polyamine, Glycine betaine, Mannitol

**Protective proteins:** LEA, Chaperone, Heat shock protein

**ROS-scavenging proteins:** Lipid peroxide, NAD+ breakdown

**Others:** Ion transport, ABA biosynthesis
Trehalose accumulation in rice plants confers high tolerance levels to drought

Regulated overexpression of *E. coli* trehalose biosynthetic genes (otsA and otsB) as a fusion gene increased drought tolerance in rice

Garg et al., 2000; PNAS 99(25):15898-903
Transgenic “Maize” drought tolerant

Agricultural biotechnology giant Monsanto has received the green light from the US Department of Agriculture to sell its transgenic drought-tolerant maize (corn) MON 87460.

Hybrid seed sold under this trademark combine a novel transgenic trait (based on the bacterial cspB gene) with the best of Monsanto's conventional breeding programme.

Drought Gard™ maize was the first commercially available transgenic (GM) drought tolerant crop released in 2013

Harrigan et al., 2009
Delay of onset of drought-induced senescence in Canola (*Brassica napus* L)

- Structural gene (IPT) encoding the cytokinin biosynthetic enzyme isopentenyltransferase was fused to a functionally active fragment of the *AtMYB32* promoter and was transformed into canola plants.
- Expression of the *AtMYB32xs::IPT* gene cassette delayed the leaf senescence in transgenic plants.

*Kant et al., 2015*
In order to provide a rational approach towards attenuation of gene expression:

We have studied various compositional and structural properties of genes and their core promoters for model plants and analysed for correlation with level and breadth of gene expression.
Eukaryotic gene architecture

Gene expression vs

✓ Gene architecture: Length and GC% of primary transcript, coding and non-coding regions were reported to be important in gene expression of higher eukaryotes
✓ Structural features of gene promoter.
Role of DNA structural features in regulation of gene expression

Canonical B-DNA duplex, has characteristic properties in Promoter region upstream of TSS:

DNA Duplex Stability
✓ – is low in promoter region
Determines ability of DNA to open up or melt.

Bendability
✓ - less bendable DNA in promoter region
leads to Nucleosome depleted/free region.

Intrinsic Curvature
✓ higher curvature in promoter regions
by intrinsic curving of DNA molecules in the absence of any external force,
Gene expression parameters

✓ Expression level: Average number of transcripts expressed

✓ Expression breadth: Number of tissues in which a gene is expressed

Cut-off

Microarray

Nelson. et al. (2009).

Expression level
- Lowly expressed
  - Less no. of transcripts
- Highly expressed
  - More no. of transcripts

Expression Breadth
- Narrowly expressed
  - Tissue specific
- Broadly expressed
  - Constitutively expressed
Bendability is intimately linked to promoter sequences (-200 to 0)

- Multivariate multiple regression model compares the relative strength of gene expression parameters as per Park et al., 2012

- Regression analysis indicates that promoters of narrowly expressed (tissue specific) genes are less stable in all plants while in monocots, constitutively expressed genes are less bendable (in terms of Dnase 1 Sensitivity)
Parameters describing gene architecture/structure

1. Length of primary transcript
2. Length of exon
3. Intron content per transcript (%)
4. Length of intron
5. Number of introns
6. Length of 5’UTR
7. Length of 3’UTR
8. GC% of primary transcript
9. GC% of concatenated intron
10. GC% of concatenated exon
11. Difference in GC% (exon - intron)
12. GC% of 5’UTR
13. GC% of 3’UTR
Variation of parameters with increase in expression

✓ Intron content of Primary transcript (%) is positively linked to both expression level and breadth
✓ Multivariate multiple regression model suggests that this feature can strongly influence expression breadth as compared to expression level
Among abiotic stress, drought is the major cause of concern for global agricultural productivity.

Changing climatic conditions demand engineering of transgenic plant to meet the required food productivity.

Genetic engineering has proved its worth in tweaking the plant’s ability to cope with various abiotic stresses by improving crosstalk across species.
Although much progress has been made through GE in controlling stress, the detailed knowledge of promoter and gene architecture would help to reach the full potential of this technology.

The promoter and gene architecture are determined by the following:

- **Intron density**: regulates the breadth of gene expression
- **AT content**: Promoters of narrowly expressed genes are less stable implying AT richness
- **Bendability**: Promoter regions of broadly expressed genes are less bendable and found to be common in plants
Acknowledgements:
Material presented has been extracted from the following publications:

- Garwe, Dahlia, Jennifer A. Thomson, and Sagadevan G. Mundree. "Molecular characterization of XVSAP1, a stress-regulated gene from Xerophyta viscosa."  
- Marais S, Thomson JA, Farrant JM, Mundree SG. XvVHA-c 1—a novel stress-responsive V-ATPase subunit c homologue isolated from the resurrection plant Xerophyta viscosa.  
- Maredza AT (2007) Isolation of the aldose reductase gene (XvAld1) from the resurrection plant Xerophyta viscosa.  
- Kurz, Matthias, Anika NS Brüning, and Erwin A. Galinski. "NhaD type sodium/proton-antiporter of Halomonas elongata."  
- Wang, Wangxia, Basia Vinocur, and Arie Altman. "Plant responses to drought, salinity and extreme temperature stress."  
- Lee, In Hye, et al. "Regulation of abiotic stress response through NtROS2a-mediated demethylation in tobacco."  
- Umezawa, Taishi, et al. "Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future."  
- Harrigan, George G., et al. "The forage and grain of MON 87460, a drought-tolerant corn hybrid, are compositionally equivalent to the non-transgenic controls."  
- Kant, Surya, et al. "Regulated expression of a cytokinin biosynthesis gene IPT delays leaf senescence and improves yield in rice."  
- Das, Priyanka, et al. "Understanding salinity responses and adopting 'omics-based' approaches to generate salinity tolerant rice varieties."
Thank You
&
Questions
✓ In monocots, similar type of profile is observed for GC% of PT, GC% of exon and difference in GC% of exon and intron
✓ Regression analysis have revealed that GC% of exon is less for highly expressed genes in arabidopsis and sorghum
Gene ontology enrichment for constitutive and tissue specific genes

<table>
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<th>Arabidopsis</th>
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- **EN** - Narrowly expressed (tissue specific)
- **EB** - Broadly expressed (constitutively expressed)

✓ GO terms were analysed using MapMan software by using hypergeometric test
✓ Tissue specific genes are enriched for the GO terms associated with secondary metabolism, hormone metabolism and stress
Increased wax synthesis improved drought tolerance

Evidences

Transcriptional factors regulating wax biosynthesis

SHINE/WIN1-AP2 ERF Transcription factors

WT

WXP1 transgenics

3d after drought stress

Zhang et al., 2005, Plant journal

(Asaph et al., 2004, Plant Cell)
Heat-tolerant basmati rice by over-expression of hsp101

- *Arabidopsis thaliana* hsp101 (Athsp101) cDNA into the Pusa basmati 1 cultivar of rice (*Oryza sativa L.*) by *Agrobacterium* mediated transformation
- Diagrammatic representation of pUH-Athsp101 construct employed for rice transformation.

Transgenics expressing AVP1 showed enhanced drought recovery in tomato.

AVP1 enhances the root growth and hence better survival at the end of stress and high recovery growth on stress alleviation.
DROUGHT and ENGINEERING
DROUGHT RESISTANCE

TYPES OF DROUGHT

1. Meteorological Drought- rainfall < 25 % of the average of the region.( <50 %- severe drought)

2. Agricultural Drought- lack of rainfall result in insufficient moisture in the root zone.

3. Hydrological Drought- extended dry period leading to marked depletion of surface water leading to drying up of reservoir, lacks stresses, rivers and fall in ground water level.
Pyramiding the drought traits

Genotype with drought traits
Root, wax, WUE

Genes coding for drought mechanism
Multiple gene construct

Increased productivity under drought

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