

## Genetic engineering for high-level tolerance to abiotic stresses through over-expression of transcription factor genes: The next frontier

Abiotic stresses (such as high salt levels, low water availability, excess water and high and low temperatures) adversely affect growth and yield of crop plants. There is a great deal of urgency in improving the performance of crops against such stress factors. During the past five years (1993–98), a range of genes [such as those linked to osmoregulation and chaperoning activities, unsaturase, antifreeze protein (AFP) genes and late embryogenesis abundant (LEA) protein genes] have been employed for raising stress-tolerant plants and the success has been to varying extents<sup>1</sup>. However, high-level tolerance against abiotic stresses still remains a major challenge<sup>1</sup>. A general criticism against genetic engineering for tolerance to abiotic stress factors has been that since response of plants to these stresses is often multi-genic, it is not possible to affect the whole cascade of cellular changes when single genes are employed. If so, expression of the entire battery of stress-responsive genes such as genes for different heat shock proteins (HSPs; induced as a response to high temperature stress) or genes for cold-regulated proteins (COR; induced as a response to low temperature stress) would have a greater beneficial effect on stress tolerance than the individual genes. This possibility has been recently tested and proven to be far more successful<sup>2,3</sup>.

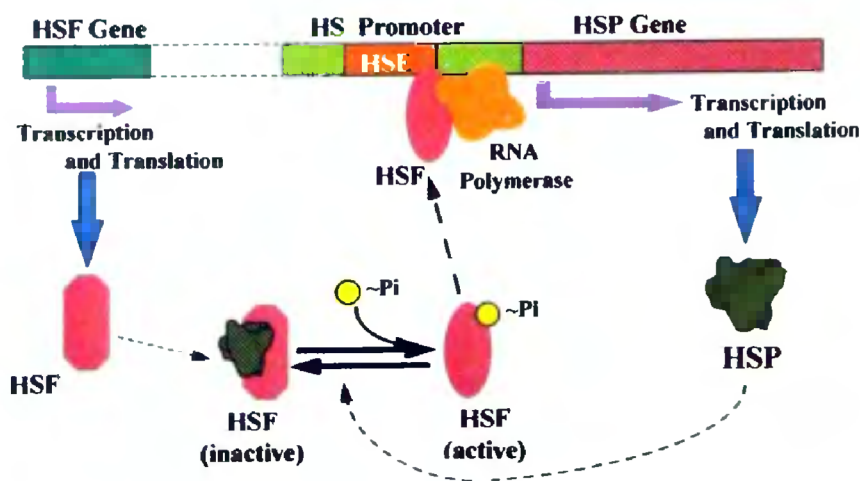
Basic molecular biology research has established that the expression of a given gene is governed by the promoter sequence present mostly at the 5' end of the gene. The promoter sequences determine the strength of the expression (i.e. strong or weak expression) as well as provide specificity to the pattern of gene expression (temporal and/or spatial). For instance, regulation of heat shock (HS) genes (referred to as *hs* or *hsp* genes) is mediated by a core DNA sequence called heat shock element (HSE), located in the promoter region of the *hs* genes (Figure 1). Presence of at least three five base pair modules (nGAAn) arranged as contiguous repeats – nGAAnnTTCnnGAAn – is the key feature of HSEs. Likewise, regulation of *cor* genes is mediated by *cis*-acting

CRT (C-repeat)/DRE (drought-responsive element) sequence that stimulates transcription in response to low temperature (and water stress)<sup>4</sup>. The *cis*-acting promoter sequences interact with specific proteins for their activation. Such proteins are generally termed as transcription factors. For the regulation of HS promoter, positively-acting transcription factors termed heat shock factors (HSFs) have also been identified that bind specifically to HSEs<sup>5</sup>. For the regulation of *cor* genes, CBF1 (CRT/DRE binding factor 1) is implicated to be the gene regulator<sup>6</sup>.

While the precise interactions between the *cis*-acting DNA sequences and the *trans*-acting protein factors are still being looked into, a new level of hierarchy has emerged for controlling expression of stress-responsive genes. This hierarchy suggests that the stress-responsive genes may be over-expressed through over-expression of the transcription factor genes. The novelty as well as importance of this approach lies in the fact that the *cis*-acting promoter sequences of different stress-responsive genes (which are induced as a response to the same stress factor) are similar, and thus can be gov-

erned at the same time through manipulation of the transcription factor genes. For instance, *cis*-acting CRT sequence is present in the promoters of multiple *cor* genes including those encoding COR15a, COR78 and COR6.6 proteins<sup>2</sup> and *cis*-acting HSE sequence is present in almost all *hs* genes sequenced so far<sup>5</sup>.

Banking on this background, Jaglo-Ottosen *et al.*<sup>2</sup> have produced transgenic *Arabidopsis* plants that over-express CBF1. This was achieved by placing a cDNA encoding CBF1 under the control of strong cauliflower mosaic virus (CaMV) 35S promoter and transforming the chimeric gene into *Arabidopsis*. Specific transformed line exhibiting higher level of accumulation of CBF1 corresponding transcript, also showed greater than normal amounts of COR6.6, COR15a, COR47 and COR78 corresponding transcripts without a low temperature stimulus. Importantly, it was found that CBF1 over-expression increased the tolerance of plants to freezing stress. According to these authors<sup>2</sup>, this scheme may well be a generalized way for improving freezing stress tolerance because CRT/DRE DNA regulatory elements have a widespread occurrence.



**Figure 1.** A model for the regulation mechanism for transcription of HSPs. Induction of *hs* gene transcription occurs when HSF binds to HSE upstream from the *hs* gene. Activation of HSF is related to phosphorylation of the HSFs and releasing them from binding to a HSP during HS. The response of HSP gene transcription to HS is a fine-tuned process that opens up many possibilities for genetic engineering. HS: Heat shock; HSP: Heat shock protein; HSE: Heat shock element; HSF: Heat shock factor.

Almost similar approach has been used by Lee *et al.*<sup>3</sup> for producing thermo-tolerant *Arabidopsis* plants. In this work, the authors showed that ATHSF1 is a HSF of *Arabidopsis* that is constitutively expressed but its activity for DNA binding, trimer formation and transcriptional activation of *hs* genes is repressed at normal temperatures. They were able to de-repress the HSF function by experimental means which led to constitutive expression of HSPs at normal temperatures. The level of basic thermotolerance of transgenic *Arabidopsis* plants, making constitutively higher level of HSPs was found to be significantly enhanced.

These two reports<sup>2,3</sup> present a new milestone as they show that by changing the expression of the transcription factor genes, it should be possible to alter levels of several target genes at the same time. This would definitely enhance the resis-

tance levels compared to single gene manipulations. It is possible that the multigenic effect will not remain a bottleneck for engineering plants to abiotic stresses. For this approach to work further, one would have to isolate, clone and characterize more transcription factor genes as till date there is insufficient information on this aspect. Further research in this area is expected to open up vast possibilities in genetic engineering of crops for high level resistance against abiotic stresses.

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ANIL GROVER  
NEETI SANAN  
CHANDAN SAHI

Department of Plant Molecular Biology,  
University of Delhi South Campus,  
Benito Juarez Road, Dhaula Kuan,  
New Delhi 110 021, India

## OPINION

### Information technology: What it means for science communication in developing countries\*

Subbiah Arunachalam

In an interview she gave to a British newspaper immediately after winning the Nobel Prize for literature, Princeton University professor Ms Toni Morrison said that it seemed as if writing about the life and sensibilities of the Blacks did not really count. It was not thought important enough to merit attention. It was peripheral.

It is the same with doing science (or working in any other area of scholarly pursuit) in the developing countries. One's work goes unnoticed. One who works under adverse conditions in the developing countries needs to achieve a lot more to win some recognition than those who work under much better conditions in the developed countries. Not surprising. After all, we live in an unequal world. Imme-

diately following the Prague conference of biomedical editors (September 1997), *New Scientist* commented in an editorial (1 November 1997) that when it came to choosing manuscripts for publication, editors of reputed international journals would more likely select the one from Harvard in preference to the one from Hyderabad. Even though both manuscripts may be of comparable quality. Harvard any day is a safer bet than Hyderabad!

Technology tends to exacerbate this inequality and further marginalize scientists on the periphery. The Internet, or for that matter any technology, does not come without its attendant problems. History has repeatedly shown that technology inevitably enhances existing inequalities. Take for example, scientific research in India. It is very important for researchers to get to know what is happening around the world as well as to let others know what they are doing. Information is key to the growth of knowledge, and dissemination of information is crucial for the

scientific enterprise. And information is disseminated through communication channels. In pre-Independent India, when scientists of the calibre of C. V. Raman, Meghnad Saha, J. C. Bose and S. N. Bose made their first-rate contributions to knowledge, the main vehicle for transmission of knowledge was the scholarly journal, and there were far fewer journals then than now. Scientists around the world were almost at the same level as far as accessing information was concerned. True that most journals were published in Europe and Raman and his Indian colleagues received the journal issues a few months later than their European colleagues – the time it took for the boat to cross the seas. Today there is tremendous proliferation of journals and many of them, especially those published by commercial firms, are out of reach for libraries even in the West what to talk of the poorer countries. (It is heartening to note that the Association of Research Libraries in the United States

\*Based on a talk delivered by the author at 'Science Communication for the Next Millennium: Ninth International Conference of the International Federation of Science Editors', Sharm El-Sheikh, Egypt, 7-11 June 1998.