Emerging trends in agricultural biotechnology research: Use of abiotic stress-induced promoter to drive expression of a stress resistance gene in the transgenic system leads to high level stress tolerance associated with minimal negative effects on growth

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Abiotic stresses (such as those imposed by excess salts, reduced water supply leading to drought stress, excess water leading to submergence and anoxia stress, sub-optimal ambient temperature leading to low temperature stress, supra-optimal ambient temperature leading to high temperature stress, oxidative stress caused by different abiotic stresses in conjunction with high light intensity, heavy metal stress, air pollutants stress, etc.) negatively affect processes associated with biomass production and grain yield, in almost all major field-grown crops. In recent years, plant genetic engineering science has successfully risen to the challenge of producing plants tolerant to several abiotic stresses. A host of genes encoding different structural and regulatory proteins have been employed over the past 5–6 years, for production of a range of abiotic stress-tolerant transgenic plants[1–7]. The appreciation is growing that the usage of regulatory genes is a more effective approach for producing stress-tolerant plants. This is based on the observations that single regulatory gene leads to altered expression of a number of different downstream structural genes, thus leading to a wide-arrayed altered response[8–10]. Liu *et al.*[11] isolated cDNA encoding DRE (dehydration responsive element)-binding proteins, DREB1A and DREB2A, and showed that both of these proteins specifically bind and activate transcription of genes containing the DRE sequences in *Arabidopsis*. This group was then prompted to over-express cDNA of DREB1A under the control of 35S

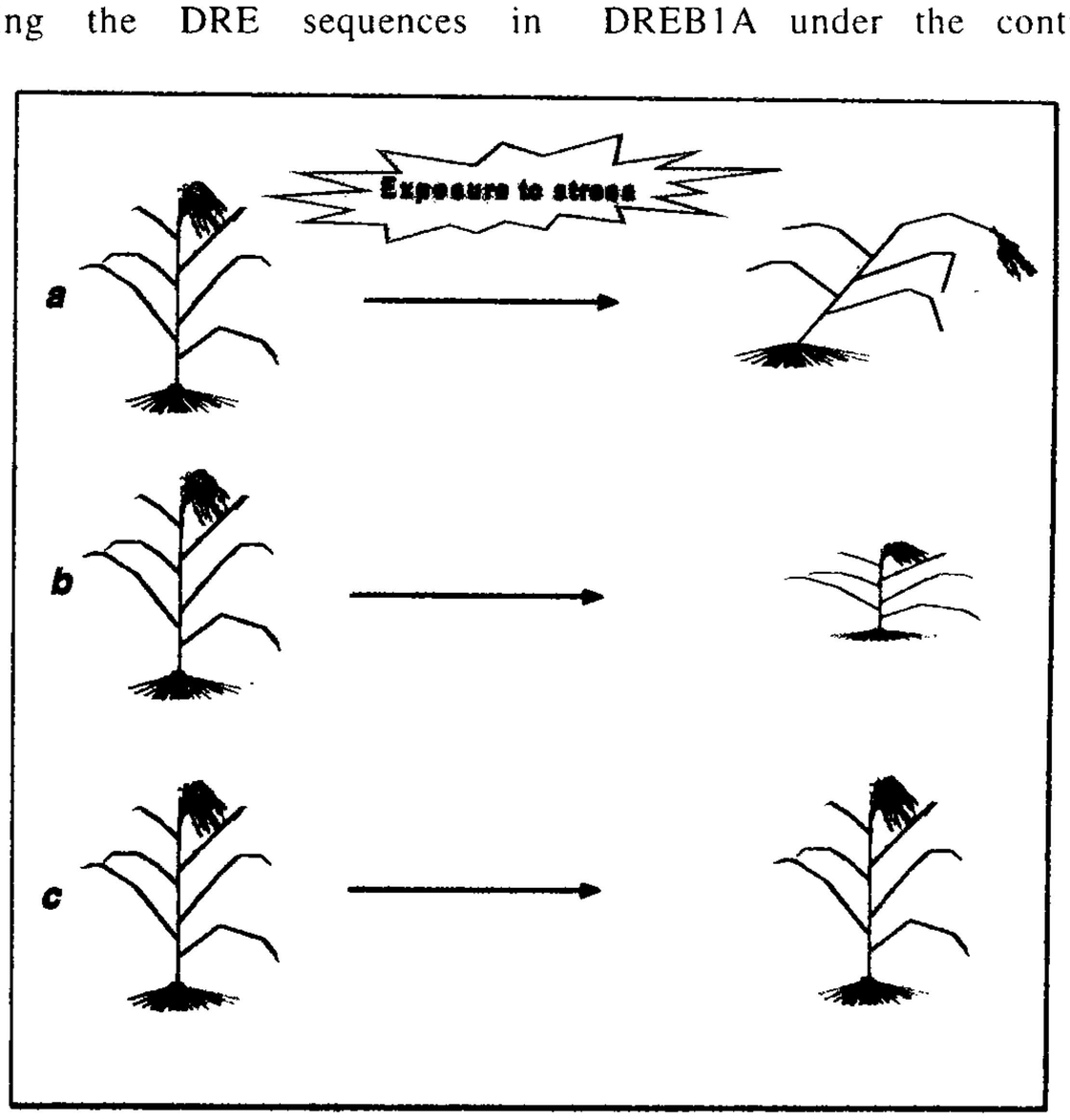

Figure 1. A model showing how the abiotic stress-induced promoter is useful in production of abiotic stress-tolerant transgenic plants. Three possible options shown above are as follows: ***a***, Non-transgenic plant exposed to stress lacks the requisite expression of the stress tolerance gene and therefore succumbs; ***b***, Transgenic plant expressing the requisite stress tolerance gene driven by a constitutive promoter overcomes the injurious effects of stress but the plant is short with conspicuous phenotypic differences; ***c***, Transgenic plant expressing the requisite stress tolerance gene driven by stress-induced promoter overcomes the injurious effects of stress and additionally is phenotypically similar to non-transgenic counterpart in terms of height, etc. The option *c* is the most desired research goal.

CaMV promoter in order to generate freezing and dehydration tolerance in *Arabidopsis*. There was indeed high expression of the stress-inducible genes coupled to increased tolerance to salt, drought and freezing stresses in the resulting transgenics; however, these effects were associated with phenotypic dwarfing of the transgenic progenies. This unwanted dwarfing of the transformed plants was thought to be due to the constitutive expression of the transgene.

The expression of a protein in amounts more than normal and at stages when it is not needed (i.e. controlled conditions) is unnecessary and taxing on the energy reserves of the cell. These options are shown graphically in Figure 1. Work on inducible promoters in plant genetic engineering research has been pursued to a great extent and a wealth of information has been delineated on promoters which are induced by anaerobic stress, high temperature stress, low temperature stress and salt stress[12]. Table 1 shows selected examples of *cis*-acting sequences which play an important role in stress-associated induction of promoters in response to different abiotic stresses (for want of space the list of references is kept small in this article). However, it has been found that most of the stress-induced promoters have poor strength of expression when compared to constitutive promoters[13]. It is therefore important that fine manipulations are carried out in such a way that the strength of stress-responsive promoters is increased without any negative impact on their induction patterns. There is also a need that such promoters are used in vectors which have all the other advantages for routine plant transformation work. There is some work on optimizing vectors with heat shock elements, anoxia-responsive elements and ABA responsive elements[14–16] but it is yet to come to the stage of routine usage.

Way back, Yamaguchi-Shinozaki *et al.*[17] isolated *A. thaliana* cDNA [referred to as dehydration-responsive (*rd*) 29 cDNA], the transcript for which showed rapid induction upon water stress. The levels of *rd29* mRNA changed differently in response to dehydration, low temperature, salt stress or exposure to ABA[18,19]. Corresponding to *rd29* cDNA, two genes namely *rd29a* and *rd29b*, were cloned from *A. thaliana*[18]. These genes were differentially induced under conditions of dehydration, low temperature, high salt or treatment with exogenous ABA. It was later found that *rd29a* has at least two *cis*-acting elements, one involved in the ABA-associated response to dehydration and the other induced by changes in osmotic potential, and the *rd29b* contains at least one *cis*-acting element that is involved in ABA-responsive, slow induction. A novel *cis*-acting DRE containing 9 bp, TACCGACAT, involved in the first rapid response of *rd29a* to conditions of dehydration or high salt, was identified[20]. Further studies showed that TACCGACAT element is essential for the regulation of dehydration-responsive gene expression and is found in the promoter regions of other dehydration and cold-stress inducible genes[21,22]. With this background, Kasuga *et al.*[23] have recently over-

Table 1. Comments on the regulatory elements present in up-stream promoter region of selected abiotic stress-responsive genes in plants

Stress agent	Gene	Comments on the regulatory elements
Water stress + ABA	*rd29a*	DRE with TACCGACAT sequence responsible for drought-responsiveness. ABRE-like element responsible for ABA-dependent induction of this gene.
	rab28	ABRE with CCACGTGGC sequence responsible for ABA-responsiveness. This gene is induced by ABA and water stress.
	rab17	ABRE has PyACGTGGC sequence.
	hva22	ABA responsive complex (ABRC) has two components namely ABRE3 (GCCACGTACA) or G-box and a novel coupling agent CE1 (TGCCACCGG).
	rab16b	ABRE has AGTACGTGGC sequence in motif I and GCCGCGTGGC sequence in motif II.
Low temperature stress	*cor15a*	Three sequence motifs G-box core elements (CACGTG), G-box related sequence (TACGTG) and C repeat (TGGCCGAC) involved in regulating gene expression in response to cold, drought and ABA.
	cor6.6	Three G-box elements with sequence CACGTG regulate gene induction by ABA, temperature, osmoticum and dehydration.
	kin1	Three G-box elements as found in *cor15a* (as shown above) but A replaced with T, regulate gene expression by low temperature, osmoticum, ABA and dehydration.
	bn115	Low temperature responsive element (LTRE) with 5-bp core sequence CCGAC required for cold induction of the gene.
High temperature stress	*hsp17.5E*	Heat shock elements (HSEs) homologous to HSE of *Drosophila* (CT–GAA–TTC–AG–) and additional upstream AT-rich elements are required for the efficient expression of the gene.
	hsp17.3B	HSE sequences and three upstream sequences (CCAAT box elements) required for enhanced expression of the gene.
	hsp17.6L	Multiple overlapping HSEs responsible for heat-inducibility. Also, AT-rich elements present which modulate the strength of the promoter.
Anaerobic stress	*adh1*, *adh2*, *aldolase*, *ldh1*, *pdc1*	Anoxia-responsive element (ARE) with GC and GT motifs responsible for the anaerobic induction of these genes.
	gapC4	ARE with the core sequence GGTTT and another motif with the nucleotide sequence ACGGTCCA responsible for anaerobic induction.

expressed DREB1A cDNA in *A. thaliana* under the control of *rd29a* promoter. It was seen that over-expression of the cDNA encoding DREB1A in transgenic plants activated the expression of many of the stress tolerance genes under normal growth conditions and resulted in improved tolerance to drought, salt loading and freezing stress. However, use of the strong 35S CaMV promoter to drive expression of DREB1A also resulted in severe growth retardation under normal growing conditions. In contrast, expression of DREB1A from the stress inducible *rd29a* promoter gave rise to minimal negative effects on plant growth while providing an even greater tolerance to stress conditions than did expression of the gene from the CaMV promoter. This outstanding piece of work shows that persistence with the same research theme pays higher dividends. The group of Kazuko Yamaguchi-Shinozaki and Kazuo Shinozaki (Institute of Physical and Chemical Research, Tsukuba Life Science Center, Japan) is credited with the isolation of *rd29* cDNA clones, the corresponding genomic clones, elucidation of the promoter elements, isolation of cDNA of the protein factors which bind to *rd29a* promoter, raising of transgenics with the DREB1A cDNA using 35S CaMV promoter and finally production of transgenics in which DREB1A cDNA is driven by *rd29a* promoter. This work has two main plus points namely (1) that single regulatory gene leads to multiple resistance against different abiotic stresses (an outcome which is shared with some of the earlier reports[8,9]) and (2) more importantly, the stress resistance-associated gene will be more useful to the *trans*-host if it is driven by stress-induced promoter rather than by a strong constitutive promoter.

It is important that some lessons are taken from this work for future research. The work on identification, isolation and cloning of abiotic stress-related regulatory genes is still in infancy. However, a number of different stress-responsive genes have been identified recently[7]. From the genomic clones of these genes, attempts must be made for the characterization of the promoter regions, in particular for the characterization of *cis*-acting regulatory sequences. The isolation of genes encoding transcription factors that interact with the *cis*-acting regulatory sequences is the key point. The transcriptional factor genes are normally expressed at very low levels[24]. Therefore, techniques of subtractive hybridization, cold-plaque screening, differential display of mRNAs and South-Western need to be exensively employed for isolation of the transcriptional factor genes. There is certainly a need to bring higher-level sophistication in gene isolation methods so that cDNA clones of minutely expressed mRNAs are selectively picked up. The future stress biotechnology research will hopefully see more of such reports in which high strength and stress-induced expression of the transgenes are combined.

1. Grover, A., Pareek, A. and Maheshwari, S. C., *Proc. Indian Natl. Sci. Acad.*, 1993, **B59**, 113–127.
2. Grover, A., Pareek, A., Singla, S. L., Minhas, D., Katiyar, S., Ghawana, S., Dubey, H., Agarwal, M., Rao, G. U. and Rathee, J., *Curr. Sci.*, 1998, **75**, 689–696.
3. Grover, A., Sanan, N. and Sahi, C., *Curr. Sci.*, 1998, **75**, 178–179.
4. Khanna-Chopra, R. and Sinha, S. K., *Curr. Sci.*, 1998, **74**, 25–34.
5. Dhaliwal, H. S., Kawai, M. and Uchimiya, H., *Plant Biotechnol.*, 1998, **15**, 1–10.
6. Grover, A., *Curr. Sci.*, 1999, **76**, 136–137.
7. Grover, A., Sahi, C. and Sanan, N., *Plant Sci.*, 1999, **143**, 101–111.
8. Lee, J. H., Hubel, A. and Schoffl, F., *Plant J.*, 1995, **8**, 603–612.
9. Jaglo-Ottosen, R., Gilmour, S. J., Zakr, D. G., Schabenberger, O. and Thomashow, M. F., *Science*, 1998, **280**, 104–106.
10. Pardo, J. M., Reddy, M. P., Yang, S., Maggio, A., Huh, G-H, Matsumoto, T., Coca, M. A., Paino-D'Urzo, M., Koiwa, H., Yun, D-J., Watad, A. A., Bressan, R. A. and Hasegawa, P. M., *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 9681–9686.
11. Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K., *Plant Cell*, 1998, **10**, 1391–1406.
12. Reynolds, P. H. S. (ed.), *Inducible Gene Expression in Plants*, CABI Publishing, Oxon, UK, 1999.
13. Holtorf, S., Apel, K. and Bohlmann, H., *Plant Mol. Biol.*, 1995, **29**, 637–646.
14. Ainley, W. M. and Key, J. L., *Plant Mol. Biol.*, 1990, **14**, 949–967.
15. Olive, M. R., Walker, J. C., Singh, K., Ellis, J. G., Llewellyn, D., Peacock, W. J. and Dennis, E. S., in *Plant Molecular Biology 2* (eds Hermann, R. G. and Larkins, B.), Plenum Press, New York, 1991, pp. 673–684.
16. Su, J., Shen, Q., Ho, T-H. D. and Wu, R., *Plant Physiol.*, 1998, **117**, 913–922.
17. Yamaguchi-Shinozaki, K., Koizumi, M., Urao, S. and Shinozaki, K., *Plant Cell Physiol.*, 1992, **33**, 217–224.
18. Yamaguchi-Shinozaki, K. and Shinozaki, K., *Mol. Gen. Genet.*, 1993, **236**, 331–340.
19. Yamaguchi-Shinozaki, K. and Shinozaki, K., *Mol. Gen. Genet.*, 1993, **238**, 97–105.
20. Yamaguchi-Shinozaki, K. and Shinozaki, K., *Plant Cell*, 1994, **6**, 251–264.
21. Iwasaki, T., Kiyosue, T., Yamaguchi-Shinozaki, K. and Shinozaki, K., *Plant Physiol.*, 1997, **115**, 1287.
22. Wang, H., Datla, R., Georges, F., Loewen, M. and Cuter, A. J., *Plant Mol. Biol.*, 1995, **28**, 605–617.
23. Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K., *Nature Biotech.*, 1999, **17**, 287–291.
24. Latchmann, D. S. (ed.), *Gene Regulation: A Eukaryotic Perspective*, Stanley Thornes Publishers Ltd., UK, 1998.

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