Addressing abiotic stresses in agriculture through transgenic technology[§]

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Abiotic stresses in agriculture – The problem

Agricultural production in Asia, particularly in India, has increased considerably during the last three decades. This has happened largely due to the development and large-scale cultivation of new higher-yielding dwarf varieties, increase in area under such varieties and greater applications of water and nutrients. This increase in food production has made the Asian region self-sufficient and contributed tremendously to food security. Despite surplus buffer stocks currently available in many parts of South-Asia, it is projected that food security of this region may again be at risk shortly due to increasing population and pressure for alternate land uses. Indian subcontinent is now home for almost one quarter of the world population. It is projected that about 3.8 billion more people will be added to the world's population by 2050. By this time, India's population is expected to grow to 1.6 billion, making it the most populous country of the world.

This rapid and continuing increase in population implies a greater demand for food. It is projected that by 2010 our food grain demand will be 246 million tons and 294 million tons by 2020 as against our current production level of 208 million tons[1]. Demand for vegetables, fruits, meat and other animal products will also rise sharply. Although the world as a whole may still have sufficient food for everyone, the food will need to be produced where needed due to socio-economic and political compulsions. In India, food will have to be produced from same or even shrinking land resource because there is no additional land available for cultivation.

Despite the development of impressive irrigation potential, which ensured food security of India during last three decades, agriculture in India is still considerably affected by climatic variability. Droughts have been frequent in different parts of India throughout its history, and are responsible for many famines, rural poverty and migration (which still occur although their geographical spread and impact has been somewhat contained). Similarly, temperatures, wind velocity and humidity during critical stages are known to significantly affect food production due to their effects on various crop growth and yield processes, pest incidences and epidemics and demand on irrigation resources.

The increased demand for food can no longer be met only by higher yields from irrigated areas. Greater efforts are needed today to understand and enhance the contribution of rainfed areas to overall agricultural production by developing and applying location-specific technologies. For example, almost 27 million hectares of the rice area in Eastern India is rainfed and is exposed to abiotic stresses such as drought, floods and poor soil fertility. The average yields of the region are lower than the national average. It is these large areas that have to be tapped in future to increase production. In general, the potential productivity of most crops is much higher than the average yields in farmer's field[2]. Most of these gaps are due to environmental factors and are difficult to manage[3].

Besides drought, the other major impediments to increased crop production are unfavourable climatic and soil conditions resulting in salt stress, low and high temperature stress, flooding stress, chemical stress, oxidative stress and other related stress types. There is hardly a landmass in India, which is not influenced by one or the other of these stress factors. In fact, most of these factors co-occur resulting in a compound effect. The drought stress is mostly accompanied by high temperature stress, salt stress is often associated with water stress and low temperature stress is associated with drought stress. The contribution due to osmotic stress is a common denominator in water stress, salt stress and low temperature stress. Likewise, the contribution due to oxidative damage is a common factor in stresses caused by excess light, excess or shortage of water, and low and high temperatures.

In recent years, there has been a general increase in extreme events including floods, droughts, forest fires and tropical cyclones in the Asian continent. A severe super-cyclone with winds of up to 250 km/h that crossed

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[§]We wish to dedicate this article to our beloved teacher late Professor S. K. Sinha who made tremendous contributions to the physiology and biochemistry of abiotic stresses on crop plants.

the Orissa coast in India on 29 October 1999 was perhaps the worst cyclone of the century, responsible for as many as 10,000 deaths, for rendering millions homeless, and for extensive property damage. Floods, landslides and storm surges caused by tropical cyclones have killed scores of people in Japan, Vietnam and China in recent past. Shortage of onions and potatoes in 1998 and gluts of onions, potatoes, rice and wheat in 2000 in India, was largely due to variable climatic conditions.

Over the past few decades, man-made changes in the climate of the earth due to the multifarious activities linked to development have become the focus of scientific and social attention. The most imminent of climatic changes of the earth is the increase in the atmospheric temperatures due to increased levels of CO_2 and other greenhouse gases. The CO_2, methane and nitrous oxides concentrations were 280 ± 6 ppm, 700 ± 60 ppb and 270 ± 10 ppb respectively between 1000 and 1750 AD. Currently, these values are 368 ppm, 1750 ppb and 316 ppb respectively. The quantity of rainfall and its occurrence have also become more uncertain. In certain places, climatic extremes such as droughts, floods, timing of rainfall and melting of snow have also increased. The sea level has risen by 10–20 cm with regional variations. Similarly, snow cover is also believed to be gradually decreasing. These changes were primarily due to the combustion of fossil fuel and land-use changes. The 1990s were, on an average, the warmest decade of the earth since instrumental measurement started in 1860s and the 1900s the warmest century during the last 1000 years. The seven warmest years globally in the instrumental record have occurred in 1990s. The global mean annual temperatures at the end of the 20th century are almost 0.7°C above those recorded at the end of the 19th century. Even at local level, these warming trends are becoming discernible. Analysis of temperature data of last thirty years indicates a slight rising trend in temperature in North-Western India. This may partly be responsible for the observed yield decline in intensive rice–wheat systems practiced in the region[4]. The mean temperature in India is projected to increase by 0.1 to 0.3°C in kharif and 0.3 to 0.7°C during rabi by 2010 and to 0.4 to 2.0°C during kharif by 2070 and to 1.1 to 4.5°C by rabi season in 2070 (ref. 5). Such a global climatic change will affect agriculture through its direct and indirect effects on crops, soils, livestock and pests. An increase in temperature can reduce crop duration, increase crop respiration rates and alter photosynthate partitioning to economic products. It is projected that mean rainfall may not change by 2010 during kharif as well as rabi seasons but an increase of up to 10% during kharif and by $\pm$ 10% during rabi by 2070 is expected[5]. At the same time, there is an increased possibility of climatic extremes such as the timing of onset of monsoons, intensities and frequencies of droughts and floods. Under such a climate change scenario, the onset of summer monsoon over India is projected to be delayed and often uncertain. This will have a direct effect not only on the rainfed crops but would also cause water storage thereby putting constraints on water availability for irrigation. Since availability of water for agriculture would have to face tremendous competition with other uses of water, agriculture in future would come under greater pressure[6,7].

In totality, practically all soil processes important for agriculture are directly affected in one way or other by abiotic stresses. Changes in precipitation patterns and amount and temperature can influence soil water content, runoff and erosion, soil workability, soil temperature, salinization, soil biodiversity, organic carbon content and nitrogen content. Vast areas suffer from drought at some stage of growth cycle. In some cases, crops suffer from floods when the crop is submerged under water for up to ten days. Acidic soils are a worldwide phenomenon. Agricultural production on acidic soils may be severely limited by a number of nutritional deficiencies. Millions of hectares of lands otherwise suitable for agriculture are not cultivated or have low productivity due to high level of salinity. Are we preparing ourselves sufficiently to meet exigencies like these that would for sure increase in magnitude in future? Some aspects of abiotic stresses can be managed by appropriate management practices and by regional development. However, this is not the focus of this paper which deals specifically with what crop biotechnology research has to offer in this context.

Transgenics for increased abiotic stress tolerance – General considerations

While a great degree of success has been obtained in the production of herbicide-, virus- and fungal-resistant plants and plants with fortified nutritional values using transgenic tools, the same has not been the case in production of abiotic stress-tolerant crops. This is largely because of the complex genetic mechanisms that govern abiotic stress tolerance. The genes that have proven somewhat effective in providing stress tolerance using a transgenic approach belong to both structural and regulatory gene categories. The structural genes are the ones that primarily govern synthesis of enzymes involved in stress tolerance-related biochemical reactions/pathways. On the other hand, regulatory genes are the ones that govern expression of structural genes at hierarchically upstream positions such as genes that control expression of transcription factors, signal transduction components or receptor-related proteins. The selective reports on abiotic stress tolerant transgenics produced so far are shown in Table 1[8–82]. The selective websites that contain information on different aspects of molecular biology and biotechnology related to abiotic stresses are http://www.stress-genomics.org/, http://www.uoguelph.ca/~jdberg/heatshock.html and http://www.plantstress.com (for more details on abiotic stress molecular biology and biotechnology research, readers can refer to several other publications from our group[83–100]).

Table 1. Selective reports on production of abiotic stress-tolerant transgenic crops

Gene	Protein	Source	Cellular role (s)	Trans-host	Promoter used	Comments	Reference
			A. Regulatory genes				
Transcription factor genes							
abi3	Abscisic acid-induced protein	*A. thaliana*	Transcription factor	*A. thaliana*	CaMV 35S	Transformants appeared to modulate low temperature-induced freezing tolerance.	Parcy *et al.*[8]
abi3	Abscisic acid-induced protein	*A. thaliana*	Transcription factor	*A. thaliana*	CaMV 35S	Marked increase in expression of low temperature-induced freezing tolerance accompanied by up-regulation of RAB18, LTI129, LTI130 and LTI178.	Tamminen *et al.*[9]
alfin1	Member of Zn finger family of proteins	*M. sativa*	Transcription factor	*M. sativa*	CaMV 35S	Transformants overexpressing *alfin1* showed salinity tolerance comparable to the NaCl tolerant plants.	Winicov and Bastola[10]
at-hsf1	Heat shock transcriptional factor 1	*A. thaliana*	Transcription factor	*A. thaliana*	CaMV 35S	Transformants exhibited thermotolerance and constitutive expression of the *hsp* genes at normal temperature.	Lee *et al.*[11]
cbf1	CRT/DRE binding factor	*A. thaliana*	Transcription factor	*A. thaliana*	CaMV 35S	Transformants showed regulation of several *cor* genes at the same time and showed freezing tolerance.	Jaglo-Ottosen *et al.*[12]
cbf3	CRT/DRE binding factor	*A. thaliana*	Transcription factor	*A. thaliana*	CaMV 35S	Transformants as in the case of *cbf1* showed regulation of several *cor* genes at the same time and showed freezing tolerance. But this also increased the freezing tolerance in non-acclimatized plants.	Gilmour *et al.*[13]
dreb1A	DRE-binding protein	*A. thaliana*	Transcription factor	*A. thaliana*	rd 29 promoter	Transformants showed enhanced expression of various stress-induced genes and showed tolerance to freezing and dehydration. The dwarfed phenotype seen with the CaMV 35S promoter was not seen here.	Kasuga *et al.*[14]
dreb1 and dreb2	DRE-binding protein	*A. thaliana*	Transcription factor	*A. thaliana*	CaMV 35S	Transformants revealed freezing and dehydration tolerance but caused dwarfed phenotypes in transgenic plants.	Liu *et al.*[15]
scof-1	*Soybean cold-inducible factor-1*	*Glycine max*	Transcription factor	*A. thaliana* and *N. tabacum*	CaMV 35S	Transformants showed induction of *cor* genes and enhanced cold tolerance of non-acclimatized transgenic *Arabidopsis* and *N. tabacum*	Kim *et al.*[16]
tsi1	Tobacco stress-induced gene1	*N. tabacum*	Transcription factor	*N. tabacum*	CaMV 35S double promoter	Transformants showed marked tolerance towards salinity and salicylic acid. The transcription factor has significant homology to EREBP/ AP2 domains.	Park *et al.*[17]
Signal transduction component genes							
at-dbf2	Cell cycle regulated phosphoprotein	*A. thaliana*	Protein kinase	*A. thaliana*	CaMV 35S	Transformants showed striking tolerance to heat, salt, cold and osmotic stress upon overexpression.	Lee *et al.*[18]
Atgsk1	*Arabidopsis* homolgue of GSK3/shaggy like kinase	*A. thaliana*	Protein kinase	*A. thaliana*	CaMV 35S	Transformants showed 30–50% accumulation of Na^+ and 15–30% accumulation of Ca^{2+} in vacuoles and also showed induced expression of NaCl stress-responsive genes *AtCP1*, *RD29A* and *CHS1* in the absence of NaCl stress.	Piao *et al.*[19]
cnb1	Calcineurin B 1	*S. cerevisiae*	Ca^{2+}-binding protein	*N. tabacum*	CaMV 35S	Transformants showed substantial NaCl tolerance by coexpression of the catalytic and the regulatory subunits.	Pardo *et al.*[20]
Oscdpk7	Calcium-dependent protein kinase	*O. sativa*	Protein kinase	*O. sativa*	CaMV 35S	Overexpression showed induction of some stress responsive genes in response to salinity/drought but not cold.	Saijo *et al.*[21]

(**Table 1.** *Cont.*)

Gene	Protein	Source	Cellular role (s)	Trans-host	Promoter used	Comments	Reference
			B. Structural genes				
Detoxification component genes							
apx3	Ascorbate peroxidase	*A. thaliana*	Putative peroxisomal membrane-bound ascorbate peroxidase	*N. tabacum*	Dual CaMV35S promoter with a terminator	Transformed plants showed increased protection against oxidative stress especially in the peroxisomes but not in chloroplasts.	Wang and Allen[22]
hvapx1	Ascorbate peroxidase	*H. vulgaris*	Peroxisomal ascorbate peroxidase involved in thermo-tolerance	*A. thaliana*	CaMV35S	Transformants were significantly more tolerant to heat stress compared to wild type.	Shi *et al.*[23]
gr	Glutathione reductase	*E. coli*	A component of the oxygen-scavenging system	*N. tabacum*	CaMV 35S	Transformants showed 3-fold increase in photooxidative stress caused by paraquat or sulfur-dioxide.	Aono *et al.*[24]
gst/gpx	Glutathione-S-transferase and glutathione peroxidase	*E. coli*	Detoxification of herbicides and toxic substances	*N. tabacum*	CaMV 35S	Transformants over-expressing GST/GPX showed stimulated seedling growth under chilling and salt stress.	Roxas *et al.*[25]
sat	Serine acetyl transferase	*E. coli*	Glutathione biosynthesis	*N. tabacum*	Artificial chimeric octopine-mannopine promoter with chloroplastic transit peptide	Transformants showed several fold higher SAT activity resulting in resistance to oxidative stress.	Blaszczyk *et al.*[26]
sod	Superoxide dismutase	*N. plumbaginifolia* *P. sativum*	Dismutation of toxic reactive oxygen intermediates	*M. sativa*	CaMV 35S	Transformants showed increased regrowth after freezing stress.	McKersie *et al.*[27]
sod	Superoxide dismutase	*A. thaliana*	Dismutation of toxic reactive oxygen intermediates	*N. tabacum*	CaMV35S with duplicated enhancer and a terminator	Transformants showed 20% higher photosynthetic activity during chilling compared to untransformed plants.	Sen Gupta *et al.*[28]
fe-sod	Fe-Superoxide dismutase	*A. thaliana*	Dismutation of reactive oxygen intermediates in chloroplasts	*N. tabacum*	CaMV 35S with chloroplastic and mitochondrial transit peptide	Transformants were more protected towards damage due to superoxide radicals.	van Camp *et al.*[29]
fe-sod	Fe-Superoxide dismutase	*A. thaliana*	Dismutation of reactive oxygen intermediates in chloroplasts	*Zea mays*	CaMV 35S	Transgenic tobacco plants express enhanced oxidative stress tolerance in chloroplasts.	van Bruesegem *et al.*[30]
fe-sod	Fe-Superoxide dismutase	*N. plumbaginifolia*	Dismutation of reactive oxygen intermediates in chloroplasts	*M. sativa*	CaMV 35S with a chloroplastic transit peptide	Transformants showed increased Fe-SOD activity, which was associated with increased winter survival.	McKersie *et al.*[31]
mn-sod	Mn-Superoxide dismutase	*N. tabacum*	Dismutation of reactive oxygen intermediates in mitochondria	*N. tabacum*	CaMV 35S with chloroplastic and mitochondrial transit peptide	Transformants expressing chloroplastic Mn-SOD provided resistance against oxidative stress generated in chloroplasts.	Bowler *et al.*[32]

(**Table 1.** *Cont.*)

Gene	Protein	Source	Cellular role (s)	Trans-host	Promoter used	Comments	Reference
mn-sod	Mn-Superoxide dismutase	*N. plumbaginifolia*	Dismutation of reactive oxygen inter mediates in mitochondria	*N. tabacum*	CaMV35S	Transgenic plants overexpressing mitochondrial Mn-SOD in chloroplasts showed enhanced resistance to MV dependent light-induced oxidative stress.	Slooten *et al.*[33]
mn-sod	Mn-Superoxide dismutase	*N. plumbaginifolia*	Dismutation of reactive oxygen inter mediates in mitochondria	*M. sativa*	CaMV 35S with a chloroplastic and mitochondrial transit peptide	Transformants showed reduced injury from water deficit stress and increased winter survival.	McKersie *et al.*[34]
mn-sod	Mn-Superoxide dismutase		Dismutation of reactive oxygen inter mediates in mitochondria	*M. sativa*	CaMV 35S with a chloroplastic and mitochondrial transit peptide	Transformants showed significantly greater survival in field under water stress and in winter.	McKersie *et al.*[35]
msalr	NADPH-dependent Aldose/aldehyde reductase	*Medicago sativa*	Detoxification	*N. tabacum*	CaMV 35S	Transformants could resist a period of water deficiency and exhibited improved recovery after rehydration.	Oberschall *et al.*[36]
Fatty acid metabolism genes							
fad7	Omega-3 fatty acid desaturase	*A. thaliana*	Causes reduction of trienoic fatty acids and hexadecatrienoic acid	*N. tabacum*	CaMV 35S	Transformants showing silencing of the gene were able to tolerate higher temperature better.	Murakami *et al.*[37]
gpat	Glycerol 3-phosphate acyltransferase	*Cucurbita* sp.	Fatty acid unsaturation	*N. tabacum*	CaMV 35S	Transformants showed less chilling damage to photosynthetic activity than the wild type.	Murata *et al.*[38]
gpat	Glycerol 3-phosphate acyltransferase	*A. thaliana*	Fatty acid unsaturation	*O. sativa*	Ubiquitin	Transformants showed greater unsaturation of fatty acids and conferred chilling tolerance to photosynthesis on rice.	Yokoi *et al.*[39]
gpat	Glycerol 3-phosphate acyltransferase	*Cucurbita* sp.	Fatty acid unsaturation	*N. tabacum*	CaMV 35S	Leaves of transformants showed more sensitivity to photoinhibition than those of the wild type plants.	Moon *et al.*[40]
Heat shock genes							
hsp17.6A	Heat shock protein 17.6A	*A. thaliana*	Molecular chaperone (*in vitro*)	*A. thaliana*	CaMV 35S	Transformants were tolerant to osmotic stress but not heat stress.	Sun *et al.*[41]
hsp17.7	Heat shock protein 17.7	*D. carota*	Heat shock protein	*D. carota*	CaMV 35S	Transformants expressed the *hsp17.7* gene in the absence of heat shock and showed increased thermotolerance.	Malik *et al.*[42]
hsp101	Heat shock protein 101	*A. thaliana*	Heat shock protein	*A. thaliana*	CaMV 35S	Transformants constitutively expressing *hsp101* tolerated sudden shifts to extreme temperature better than the controls.	Queitsch *et al.*[43]
hsp101	Heat shock protein 101	*A. thaliana*	Heat shock protein	*O. sativa*	Ubi1	Transformants expressing *hsp101* showed enhanced tolerance to high temperature.	Katiyar-Agarwal *et al.*[44]
Osmolyte biosynthesis							
betA	Choline dehydrogenase	*E. coli*	Glycinebetaine biosynthesis	*N. tabacum*	CaMV 35S	Transformants showed better survival at high salt levels than the non-transformed ones.	Lilius *et al.*[45]
bet	Choline dehydrogenase	*E. coli*	Glycinebetaine biosynthesis	*Synechococcus* sp.	CaMV 35S	Transformants showed survival of enzyme Rubisco in plants under salt stress indicating a protective role of glycine betaine to Rubisco protein.	Nomura *et al.*[46]

(**Table 1.** *Cont.*)

Gene	Protein	Source	Cellular role (s)	Trans-host	Promoter used	Comments	Reference
betA	Choline dehydrogenase	*E. coli*	Glycinebetaine biosynthesis	*N. tabacum*	CaMV 35S	Transformants showed increased stress tolerance probably due to improper protection of the photosynthetic apparatus.	Holmstrom *et al.*[47]
betB	Betaine aldehyde dehydrogenase	*E. coli*	Glycinebetaine biosynthesis	*N. tabacum*	CaMV 35S	Transformed plants showed better growth in osmotic stress conditions.	Holmstrom *et al.*[48]
codA	Choline oxidase A	*Arthrobacter globiformis*	Glycinebetaine biosynthesis	*A. thaliana*	CaMV 35S/ rbcS tr.	Transformants were tolerant to salt and cold.	Hayashi *et al.*[49]
codA	Choline oxidase A	*Arthrobacter globiformis*	Glycinebetaine biosynthesis	*O. sativa*	CaMV 35S with transit peptide guided to chloroplast and cytosol	Transformants accumulated high levels of glycinebetaine and showed increased tolerance to salt and low temperature stress.	Sakamoto *et al.*[50]
codA	Choline oxidase A	*Arthrobacter globiformis*	Glycinebetaine biosynthesis	*A. thaliana*	CaMV 35S	Transformants showed tolerance to high temperature during imbibition and germination of the seeds.	Alia *et al.*[51], Sakamoto *et al.*[52]
codA	Choline oxidase A	*Arthrobacter globiformis*	Glycinebetaine biosynthesis	*Brassica juncea*	CaMV 35S with nopaline synthase terminator and choloroplastic transit peptide	Transformed seeds showed enhanced capacity to germinate under salt stress, compared to wild type.	Prasad *et al.*[53]
ectA, *ectB*, *ectC*	L-2,4-diamino butyric acid acetyl transferase, L-2,4-diamino butyric acid transaminase, L-ectoine synthase	*Halomonas elongata*	Ectoine biosynthesis	*N. tabacum*	CaMV 35S	Transformants showed increased tolerance to hyperosmotic stress.	Nakayama *et al.*[54]
hva1	Lea protein	*H. vulgare*	Unknown	*O. sativa*	Rice actin promoter	Transformants were more tolerant to water deficit and salt stress.	Xu *et al.*[55]
imt1	Myo-inositol-*o*-methyl transferase	*M. crystallinum*	D-Ononitol biosynthesis	*N. tabacum*	CaMV 35S	Transformants were better adapted to water and salt stress.	Sheveleva *et al.*[56], Vernon *et al.*[57]
mtlD	Mannitol-1 phosphate dehydrogenase	*E. coli*	Mannitol metabolism	*N. tabacum*	CaMV 35S	Transformants showed better growth under salt stress compared to untransformed controls.	Tarczynski *et al.*[58,59]
mtlD	Mannitol-1 phosphate dehydrogenase	*E. coli*	Mannitol metabolism	*A. thaliana*	CaMV 35S	Transformants were more tolerant to salt stress than the wild type.	Thomas *et al.*[60]
mtlD	Mannitol-1 phosphate dehydrogenase	*E. coli*	Mannitol metabolism	*N. tabacum*	CaMV 35S with *rbcS3A* gene transit peptide	Transformants were more tolerant to oxidative stress.	Shen *et al.*[61]
otsA, otsB	Trehalose-6-phosphate synthetase, Trehalose-6-phosphate phosphatase	*E. coli*	Trehalose biosynthesis (osmolyte accumulation)	*N. tabacum*	CaMV 35S with double enhancer	Transformants showed increased biomass production under stress and were substantially different in morphogenesis.	Pilon-Smits *et al.*[62]
p5cs	O^1-pyrroline 5-carboxylate synthase	*V. aconitifolia*	Proline biosynthesis	*N. tabacum*	CaMV 35S	Transformants accumulated 2-fold more proline than the wild type plants and were more tolerant to water stress.	Kishor *et al.*[63], Hong *et al.*[64]
p5cs	O^1-pyrroline 5-carboxylate synthase	*V. aconitifolia*	Proline biosynthesis	*O. sativa*	AIPC (ABA-induced promoter complex) – stress inducible promoter	Transformed rice plants showed tolerance to salt and water stress.	Zhu *et al.*[65]

(Table 1. *Cont.*)

Gene	Protein	Source	Cellular role (s)	Trans-host	Promoter used	Comments	Reference
prodh	Proline dehydrogenase	*A. thaliana*	Proline biosynthesis	*A. thaliana*	CaMV 35S where the ProDH protein was reverse-fused to achieve antisense expression of the gene	The antisense transgenics were more tolerant to freezing and high salinity than wild types.	Nanjo *et al.*[66]
sacB	Levan sucrase	*A. subtilis*	Fructan biosynthesis	*N. tabacum*	CaMV 35S	Transformants were more tolerant to freezing and PEG-mediated water stress than the wild type.	Pilon-Smits *et al.*[67]
tps1	Trehalose 6-phosphate synthase	*A. thaliana*	Trehalose biosynthesis (osmolyte accumulation)	*N. tabacum*	CaMV 35S	Transformants were more tolerant to drought and salinity.	Holmstrom *et al.*[68]
tps1	Trehalose 6-phosphate synthase	*S. cerevisiae*	Trehalose biosynthesis (osmolyte accumulation)	*N. tabacum*	CaMV 35S	Transformants exhibited trehalose accumulation and improved drought tolerance.	Romero *et al.*[69]
Transporter protein genes							
ala1	Aminophospholipid ATPase 1	*A. thaliana*	P-type ATPase	*A. thaliana*	CaMV 35S	Transformants showing down regulation results in cold-affected plants that are much smaller than the wild type.	Gomes *et al.*[70]
atnhx1	Na^+/H^+ antiporter	*A. thaliana*	Vacuolar Na^+/H^+ antiporter	*A. thaliana*	supermas	Transformants showed sustained growth and development in soil water with high sodium chloride.	Apse *et al.*[71]
atnhx1	Na^+/H^+ antiporter	*A. thaliana*	Vacuolar Na^+/H^+ antiporter	*L. esculentum*	CaMV 35S	Transformants showed sustained growth in high NaCl (200 mM) concentration with no Na^+ accumulation in fruits, potentiating its use as a GM (genetically modified) crop.	Zhang and Blumwald[72]
atnhx1	Na^+/H^+ antiporter	*A. thaliana*	Vacuolar Na^+/H^+ antiporter	*Brassica napus*	CaMV 35S	Transformants showed tolerance to high salt concentrations (200 mM), but showed no change in oil seed content.	Zhang *et al.*[73]
hal1	Protein involved in regulation of K^+transport	*Saccharomyces cerevisiae*	Regulation of K^+ transport	*Lycopersicon esculentum*	CaMV 35S	Transformants showed higher level of salt tolerance and transgenics were able to retain more K^+ than controls under salt stress.	Gisbert *et al.*[74]
Others							
afa	Antifreeze protein (AFP) analogue	Synthetic	Inhibits ice growth and recrystallization	*L. esculentum*	CaMV 35S	Transformants showed inhibition of ice recrystallization	Hightower *et al.*[75]
afp	Antifreeze protein (AFP)	Synthetic	Inhibits ice growth and recrystallization	*S. tuberosum*	19S RNA promoter of CaMV	Transformants showed frost tolerance.	Wallis *et al.*[76]
atnced3	Arabidopsis thaliana 9-cis-epoxy carotenoid dioxygenase	*A. thaliana*	ABA biosynthesis	*A. thaliana*	CaMV35S	Transformants showed an increase in endogenous ABA levels and enhanced level of transcription of drought and ABA-inducible genes. They also showed a reduced transcription rate in leaves and an improvement in drought tolerance.	Iuchi *et al.*[77]
bip	Binding protein	*G. max*	Molecular chaperone involved in unfolded protein response (UPR)	*N. tabacum*	CaMV35S	Transformants were more tolerant to water stress.	Alvim *et al.*[78]

(**Table 1.** *Cont.*)

Gene	Protein	Source	Cellular role (s)	Trans-host	Promoter used	Comments	Reference
cor15a	Cold regulated gene	*A. thaliana*	Promotes freezing tolerance	*A. thaliana*	CaMV 35S	Transformants showed *in vivo* enhanced freezing tolerance of protoplasts and the chloroplasts.	Artus *et al.*[79]
gly1	Glyoxylase	*Brassica juncea*	Converts 2-oxoaldehydes into 2-hydroxy acids	*N. tabacum*	CaMV 35S	Transformants overexpressing glyoxylase 1 showed tolerance to methylglyoxal and high salt.	Veena *et al.*[80]
gpd	NAD^+ dependent glyceraldehyde -3-phosphate dehydrogenase	*Pleurotus sajor-caju*	Glycolytic pathway	*S. tuberosum*	CaMV 35S	Transformed potato plants showed salt stress tolerance.	Jeong *et al.*[81]
gs2	Glutamine synthetase	*O. sativa*	Glutamine synthesis	*O. sativa*	CaMV 35S	Transformants with overexpressed GS2 showed tolerance to salt stress.	Hoshida *et al.*[82]

While insect-, viral- and herbicide-resistant transgenic plants are being field-tested and some of them are close to release for cultivation, field-level deployment of abiotic stress-tolerant transgenics is still distant. The reports on production of abiotic stress tolerant transgenics described in Table 1 basically represent experiments carried out at a laboratory scale. There are several lacunae in production of abiotic stress-tolerant transgenics that need to be plugged to bring this science at par with other applications. Certain issues that merit immediate attention are:

1. An important aspect of transgenic technology is the regulated expression of transgenes. The promoters that have been most commonly employed in the production of abiotic stress-tolerant plants so far include the CaMV35S (mostly used for dicot crops), ubiquitin1 and actin1 promoters (used for expression of transgenes in monocot crops) (Table 1). As these promoters are constitutive, the downstream transgenes are by and large expressed in all organs and at all stages which is unnecessary as well as taxing on the energy reserves of the cell. Kasuga *et al.*[14] noted that the overexpression of the *dreb1A* transcription factor gene under the control of stress-induced *rd29A* promoter showed better phenotypic growth of the transgenic plants than the ones obtained using the constitutive CaMV35S promoter, indicating the importance of applying specific stress-induced promoters in transgenic research. However, work on stress-inducible promoters has not been pursued to a great extent. There is a strong need to obtain increased array of stress-induced promoters and to pair such promoters with the stress tolerance-related genes in the requisite cloning vectors.
2. It has been a general practice to express the transprotein in the cytoplasm of the trans-host. There is a possibility that the product of the transgene is needed in a specific cellular compartment or there may be a change in the compartmentalization of the concerned protein following stress[85]. There are limited examples wherein the constitutive promoter used for expressing a stress-related transgene was provided with a transit peptide sequence targeting the protein specifically to a given organelle[31,61]. Clearly there is a need to extend the range of expression vectors to enable expression in organelles such as chloroplast, endoplasmic reticulum, vacuole and mitochondria.
3. As there is likely to be a pressing need for multiple gene introductions to achieve abiotic stress tolerance, methods that lead to pyramiding or stacking of transgenes in the same host cell are needed. This can, for instance, be achieved if cloning vectors with different promoters (to avoid homology-based gene silencing) and selection marker genes (to individually select different genes) are available. The construction of BIBAC- type vectors that which can accommodate up to 150 kb of inserts[101] is the need of the hour.
4. Major success in the production of abiotic stress-tolerant transgenics has been achieved in model plants such as tobacco and *Arabidopsis* (Table 1) but, by and large, crops have not yet been the focus of attention. There is a clear need to introduce abiotic stress tolerance-related genes that have worked with model species into crop plants.
5. Following the initial results with primary transformants which showed that a given protein appears important in conferring stress tolerance, there is a need for extensive experimentation (taking in view issues such as segregation, production of homozygosity, analysis of expression levels, etc.) in stabilizing the transgene in the progeny of primary transformants. Also, there is a need to transfer the transgene

from the primary cultivars that are transformed into the cultivars that are locally-grown. An extensive quantum of genetical and breeding work on primary transgenics has to be carried out before the expression of the transgene is stabilized, so that specific cultivar can be bred that is acceptable to local farmers. This demands active collaboration of plant biotechnologists with plant geneticists and breeders.

6. The introduction of the transgene has to be examined in the context of the overall yield of the plant at the field-level as it is possible that a given transgene leads to stress tolerance but brings in certain traits that are not acceptable in cropping systems. For instance, there may be a penalty on biomass and yield or a change in plant phenotypic characteristics associated with increased stress-tolerance. Such an analysis needs inputs from physiologists, biochemists and geneticists. Molecular biology alone would not provide complete solution to the problem of production of abiotic stress-tolerant transgenics. While collaboration between plant molecular biologists and biochemists exists to an extent, collaboration amongst molecular biologists, crop physiologists and agronomists usually does not. The latter category of scientists is often best equipped for field-testing of the abiotic stress-tolerant transgenics. The best results can be achieved by collaboration between universities and agricultural research institutes.

Transgenics for increased abiotic stress tolerance – Indian scenario

Several groups in India are working on cellular responses triggered by abiotic stress factors on microbial, animal and plant systems. For want of space, we will be selective in presentation in this section. Gowrishankar at the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad has made pioneering contribution in the identification of several transporters including ProU (glycinebetaine uptake), ProP (proline uptake) and Kdp (K uptake) related with water stress adaptation in *E. coli* and in analysis of the transcriptional regulation of the genes encoding these transporters[102–104]. Apte's group at the Bhabha Atomic Research Centre (BARC), Mumbai has cloned several osmoresponsive genes from a marine nitrogen fixing cyanobacterium *Anabena torulosa* using substractive RNA hybridization and other recombinant DNA techniques[105,106]. Lakhotia's group at the Banaras Hindu University, Varanasi has been most active in the field of heat shock proteins in India. This group has significantly contributed to characterization of one of the unique heat shock genes, *hsrw* of *Drosophila melanogaster*, which does not encode for a protein product[107]. Recently, this group showed that *hsrw* gene regulates the activity of hnRNPs[108]. Several laboratories in India have significantly contributed towards understanding the physiology and biochemistry of plant abiotic stresses on diverse plants both at universities and research institutes. The most noteworthy amongst these are Sinha and Chopra's group at the Indian Agricultural Research Institute (IARI), New Delhi which has worked on the understanding of drought and high temperature stress responses in wheat and pulses[109–112] and Uday Kumar's group at the University of Agricultural Sciences (UAS), Bangalore, which has studied biological role of late embryogenesis abundant proteins (LEA proteins) and other related aspects[113–115]. As we wish to mainly discuss the molecular biology and biotechnology of abiotic stress responses in this article, we do not discuss biochemistry and physiology-related areas of stress biology in detail. We also exclude important contributions being made on molecular markers associated with drought stress by Shashidhar and Hittalmani's group at UAS, Bangalore[116] for the same reason.

The production of abiotic stress-tolerant transgenics in India is a relatively recent development. The issues involved in raising of abiotic stress-tolerant transgenics such as identification and cloning of new candidate genes and promoters and raising of transgenics are being looked into in different laboratories in India. Several groups have taken a lead in production of abiotic stress-tolerant transgenics. Sopory's group at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, is a long-established group dedicated towards understanding the components of calcium-mediated cellular signalling[117,118]. They have raised transgenic tobacco plants resistant against salinity stress by making use of the glyoxylase pathway. This pathway has two enzymes encoded by *glyI* and *glyII* genes and both these act co-ordinately to convert methylglyoxal to lactic acid. Transformation of tobacco with *gly1*, a calcium-binding protein, resulted in enhanced salinity and metal tolerance of transgenic tobacco plants[80]. Recent work of Singla-Pareek and Sopory has indicated that in transgenics harbouring both *glyI* and *glyII*, the two genes function in a synergistic manner and provide increased tolerance to salinity and metal toxicity in tobacco (unpublished). For increasing salt tolerance in rice, Singla-Pareek at ICGEB is aiming at introduction of vacuolar ATPase and Na^+–H^+ antiporter gene in rice. Rajam's group at the University of Delhi South Campus (UDSC), New Delhi is aiming at the generation of transgenic rice, eggplant and tobacco plants for salinity, drought and chilling tolerance through the manipulation of the pathway of polyamines and carbohydrates. This group has developed efficient regeneration and *Agrobacterium*-mediated transformation protocols for indica rice[119,120] and eggplant[121–123]. Preliminary results of this group have shown that *odc* (which encodes ornithine decarboxylase; Kumria and Rajam[124]), *adc* (which encodes arginine decarboxylase), *samdc* (which encodes S-adenosylmethionine decarboxylase) and *mltD*

(encodes mannitol-1-phosphate dehydrogenase)[125] confer enhanced tolerance to osmotic stresses. Pardha Saradhi's group at the Jamia Millia University, New Delhi (currently at University of Delhi, Delhi) transformed *codA* gene in *Brassica juncea* leading to a significant enhancement in salt tolerance[53,126]. Working in collaboration with Norio Murata's group, this laboratory has shown that ABA protects photosynthetic machinery against photodamage. Tyagi's group at UDSC has made salt-tolerant transgenics by transferring *codA* gene in indica rice plants[127]. Grover's laboratory at UDSC has made contribution towards the characterization of *hsp100* gene/protein family in rice[97,98,128–131]. This group has recently produced transgenic rice over-expressing *hsp100* and *pyruvate decarboxylase1* (*pdc1*) genes[44,132], which are being tested for their stress response. Bansal's laboratory at the National Research Centre on Plant Biotechnology (NRC on Plant Biotechnology), IARI, New Delhi is employing *osmotin*, *connexin* and *codA* genes for production of abiotic stress tolerance transgenics[133,134]. These genes have been transformed individually or in combination and the constructs have been designed so that the gene over-expresses either in the cytosol or in the plastids. This group is also involved in transformation of rice, eggplant and tobacco plants with genes involved in polyamine metabolism for resistance against osmotic stress. Majumder's group at the Bose Institute, Kolkata is focusing on metabolic engineering of pathways leading to osmoprotectant biosynthesis under stress conditions. The genes for inositol synthase from rice (RINO) and *Porteresia* (PINO) have been cloned and completely sequenced which have revealed substantial differences in the nucleotide sequences between them. The bacterially expressed protein from both these cloned genes has shown that the PINO shows a better salt-tolerant character than RINO (unpublished). Following a lead from this work, effectiveness of PINO in conferring salt tolerance under transgenic conditions is now being tested in rice, *Brassica* and tobacco. George Thomas's group at SPIC Foundation, Chennai, is involved in engineering *hal1* gene from yeast in eggplant and rice. At the same time, this group has also been trying to study the role of antiporters in salt tolerance from rice and an alga *Dunaliella*.

Apart from the transgenic work mentioned above, several groups in India are attempting to isolate novel abiotic stress-related genes through characterization of proteins by 1- and 2-dimensional protein gel electrophoresis and cDNA library screening. Grover (UDSC), Sopory (ICGEB) and Reddy (ICGEB) in a joint study have isolated 1266 cDNA clones that are associated with response of rice to salt stress and 85 of these clones have been partially sequenced[135]. On the theme of ESTs, Reddy's laboratory at the University of Hyderabad (UH), Hyderabad, has isolated and sequenced a number of cDNA clones associated with response of rice to drought stress (unpublished). Parida's group at the M.S. Swaminathan Research Foundation has been working on the identification of novel genetic combinations from the salt-tolerant mangrove species offering tolerance to coastal salinity. This group has constructed six cDNA libraries from the mangrove species *Avicennia marina* and *Porteresia coarctata* and has isolated 15 full length genes with practical implications in abiotic stress management. Catalase and superoxidedismutase have also been mobilized into tobacco, *Brassica*, *Vigna* and rice through *Agrobacterium*-mediated transformation (unpublished). Tyagi's group at UDSC has recently isolated a novel S-adenosyl-L-methionine synthetase cDNA from rice and have shown that the transcript level corresponding to this clone increases in response to salt, drought and ABA, but is not influenced by cold stress[136]. Pareek at GGS Indraprastha University (Delhi) has isolated histidine kinase (one of the possible osmosensor genes) from rice. Grover's group (UDSC) has reported a large number of transcripts/proteins that are specifically altered in rice seedlings upon exposure to different abiotic stresses[137–141]. Several stress-associated proteins from rice have also been characterized by Reddy and his colleagues at UH, Hyderabad[142,143]. This group has provided evidence for the ability of proline to stabilize the DNA double helix[144]. Apte's group at BARC has identified several polypeptides, which could serve as useful markers in the rice breeding programme[145]. The chloroplast fructose-1,6-biphosphate from rice and *Porteresia* has been studied under salinity stress by Majumder's group at the Bose Institute[146]. As already discussed, characterization of stress-induced promoters is crucial for the development of effective transgenics against abiotic stresses. Sengupta's group at the Bose Institute is characterizing ABA responsive element (ABRE) in great detail[147,148]. In their work, gel mobility shift analysis showed the presence of low level of abscisic acid responsive element (ABRE) containing DNA-binding protein in rice nuclear extract from control plants and the binding activity was found to be enhanced when nuclear extract was prepared from salt-treated rice nuclear extract. Grover's laboratory at UDSC has isolated rice *hsp100* promoter and raised transgenic rice plant with *hsp100* promoter-*gusA* gene construct which are currently being analysed (Agarwal *et al.*, unpublished).

Being a large country, India has diverse climatic and soil types, varied agriculture patterns and poor infrastructure in farming sector. There is thus an urgent need for production of abiotic stress-tolerant plants in India than anywhere else. However, the work on production of abiotic stress-tolerant plants is yet a sub-critical activity in the Indian context. With an aim to improving research efforts in this direction, the following general observations are made on the work being carried in Indian laboratories towards production of abiotic stress-tolerant crops: There is need for a greater number of dedicated

laboratories which deal solely with the production of abiotic stress-tolerant transgenic crops. Another area which requires significant inputs is the discovery of novel genes. There is a need to support on a large-scale basic research leading to identification, isolation and cloning of novel abiotic stress tolerance related genes from Indian germplasm. The availability of diversity in Indian germplasm is an enormous asset for the isolation of novel genes.

Final remarks

Considering the urgency in production of abiotic stress-tolerant transgenics, the recent success on laboratory-production of such transgenics is a welcome sign that must be further explored and strengthened in future years. For luring more young minds to this important endeavour, we suggest that there should be special emphasis on research work aiming at production of abiotic stress-tolerant transgenics by the Government of India. There should be more intense programmes dealing with isolation of new genes (using recent tools provided by genomics and proteomics studies), designing of vectors, transformation and evaluation of progenies. The quantum of funding support for these aspects must increase for meeting the objectives in this important discipline of agricultural science.

1. Kumar, *Food Demand and Supply Projections for India*, Indian Agricultural Research Institute, New Delhi, India, 1998.
2. Aggarwal, P. K., Bandyopadhyay, S. K., Pathak, H., Kalra, N., Chander, S. and Sujith Kumar, S., *Outlook Agric.*, 2000, **29**, 259–268.
3. Ramasamy, C., Rice Genetics III. Proceedings of the Third International Rice Genetics Symposium, IRRI, Manila, Philippines, 1996, pp. 978–983.
4. Aggarwal, P. K., Talukdar, K. K. and Mall, R. K., Potential yields of rice-wheat system in the Indo-Gangetic plains of India, Rice-Wheat Consortium Paper Series 10. New Delhi, India, 2000, RWCIGP, CIMMYT, pp. 16.
5. Watson, R. T., Zinyowera, M. C., Moss, R. H. and Dokken, D. J., Intergovernmental Panel on Climate Change, WMO-UNEP Rep., Cambridge University Press, UK, 1998, pp. 517.
6. Aggarwal, P. K., Roetter, R., Kalra, N., Hoanh, C. T., van Keulen, H. and Laar, H. H., *Land Use Analysis and Planning for Sustainable Food Security, with an Illustration for the State of Haryana*, IARI, New Delhi and IRRI, Philippines, 2001, p.167.
7. Aggarwal, P. K. and Mall, R. K., *Climate Change*, 2002, **52**, 331–343.
8. Parcy, F., Valon, C., Raynal, M., Gaubier-Comella, P., Delseny, M. and Giraudat, J., *Plant Cell*, 1994, **6**, 1567–1582.
9. Tamminen, I., Makela, P., Heino, P. and Tapio Palva, E., *Plant J.*, 2001, **25**, 1–8.
10. Winicov, I. and Bastola, D. R., *Plant Physiol.*, 1999, **120**, 473–480.
11. Lee, J. H., Hubel, A. and Schoffl, F., *Plant J.*, 1995, **8**, 603–612.
12. Jaglo-Ottosen, K. R., Gilmour, S. J., Zarka, D. G., Schabenberger, O. and Thomashow, M. F., *Science*, 1998, **280**, 104–106.
13. Gilmour, S. J., Sebolt, A. M., Salazar, M. P., Everard, J. D. and Thomashow, M. F., *Plant Physiol.*, 2000, **124**, 1854–1865.
14. Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K., *Nature Biotechnol.*, 1999, **17**, 287–291.
15. Liu, Q., Kasuga, M., Sakuma, Y., Abe, S., Miura, H., Yamaguchi-Shinozaki, K. and Shinozaki, K., *Plant Cell*, 1998, **10**, 1391–1406.
16. Kim, J. C. *et al.*, *Plant J.*, 2001, **25**, 247–259.
17. Park, J. M., Park, C. J., Lee, S. B., Ham, B. K., Shin, R. and Palk, K. H., *Plant Cell*, 2001, **13**, 1035–1046.
18. Lee, J. H., van Montagu, M. and Verbruggen, N., *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 5873–5877.
19. Piao, H. L., Lim, J. H., Kim, S. J., Cheong, G. W. and Hwang, I., *Plant J.*, 2001, **27**, 305–314.
20. Pardo, J. M., Reddy, M. P. and Yang, S., *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 9681–9683.
21. Saijo, Y., Hata, S., Kyozuka, J., Shimamoto, K. and Izui, K., *Plant J.*, 2000, **23**, 319–327.
22. Wang, H. and Allen, R. D., *Plant Cell Physiol.*, 1999, **40**, 725–732.
23. Shi, W. M., Muramoto, Y., Udea, A. and Takabe, T., *Gene*, 2001, **273**, 23–27.
24. Aono, M., Kubo, A., Saji, H., Tanaka, K. and Kondo, N., *Plant Cell Physiol.*, 1993, **34**, 129–135.
25. Roxas, V. P., Smith, R. K. Jr., Allen, E. R. and Allen, R. D., *Nature Biotechnol.*, 1997, **15**, 988–991.
26. Blaszczyk, A., Brodzik, R. and Sirko, A., *Plant J.*, 1999, **20**, 237–243.
27. McKersie, B. D. *et al.*, *Plant Physiol.*, 1993, **103**, 1155–1163.
28. Sen Gupta, A., Heinen, J. L., Holaday, A. S., Burke, J. J. and Allen, R. D., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 1629–1633.
29. van Camp, W., Capiau, K., van Montagu, M., Inze, D. and Slooten, L., *Plant Physiol.*, 1996, **112**, 1703–1714.
30. van Bruesegem, T., Slooten, L., Stassart, J. M., Moens, T., Botterman, J., van Montagu, M. and Inze, D., *Plant Cell Physiol.*, 1999, **40**, 515–523.
31. McKersie, B. D., Murnaghan, J., Jones, K. S. and Bowley, S. R., *Plant Physiol.*, 2000, **122**, 1427–1437.
32. Bowler, C. *et al.*, *EMBO J.*, 1991, **10**, 1723–1732.
33. Slooten, L., Capiau, K., van Camp, W., van Montagu, M., Sybesma, C. and Inze, D., *Plant Physiol.*, 1995, **107**, 737–750.
34. McKersie, B. D., Bowley, S. R., Harjanto, E. and Leprince, O., *ibid*, 1996, **111**, 1177–1181.
35. McKersie, B. D., Bowley, S. R. and Jones, K. S., *ibid*, 1999, **119**, 839–847.
36. Oberschall, A. *et al.*, *Plant J.*, 2000, **24**, 437–446.
37. Murakami, Y., Tsuyama, M., Kobayashi, Y., Kodama, H. and Iba, K., *Science*, 2000, **287**, 476–479.
38. Murata, N., Ishizaki-Nishizawa, O., Higashi, H., Hayashi, S., Tasaka, Y. and Nishida, I., *Nature*, 1992, **356**, 710–713.
39. Yokoi, S., Hogashi, S., Kishtiani, S., Murata, N. and Toriyama, K., *Mol. Breed.*, 1998, **4**, 269–275.
40. Moon, B. Y., Higashi, S. I., Gombos, Z. and Murata, N., *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 6219–6223.
41. Sun, W., Bernard, C., van de Cotte, B., van Montagu, M. and Verbruggen, N., *Plant J.*, 2001, **27**, 407–415.
42. Malik, M. K., Solvin, J. P., Hwang, C. H. and Zimmerman, J. L., *ibid*, 1999, **20**, 89–99.
43. Queitsch, C., Hong, S-W., Vierling, E. and Lindquist, S., *Plant Cell*, 2000, **12**, 479–492.
44. Katiyar-Agarwal, S., Agarwal, M. and Grover, A., *Plant Mol. Biol.*, 2003 (in press).
45. Lilius, G., Holmberg, N. and Bulow, L., *Biotechnology*, 1996, **14**, 177–180.
46. Nomura, M., Hibino, T., Takabe, T., Sugiyama, T., Yokota, A., Miyake, H. and Takabe, T., *Plant Cell Physiol.*, 1998, **39**, 425–432.
47. Holmstrom, K. O., Somersalo, S., Mandal, A., Palva, E. T. and Welin, B., *J. Expt. Bot.*, 2000, **51**, 177–185.
48. Holmstrom, K. O. *et al.*, *Plant J.*, 1994, **6**, 749–758.
49. Hayashi, H., Mustardy, A., Deshnium, P., Ida, M. and Murata, N., *ibid*, 1997, **12**, 133–142.

50. Sakamoto, A., Murata, A. and Murata, N., *Plant Mol. Biol.*, 1998, **38**, 1011–1019.
51. Alia, Hayashi, H., Sakomoto, A. and Murata, N., *Plant J.*, 1998, **16**, 155–161.
52. Sakomoto, A., Valverde, R., Alia, Chen, T. H. H. and Murata, N., *ibid*, 2000, **22**, 449–453.
53. Prasad, K. V. S. K., Sharmila, P., Kumar, P. A. and Pardha Saradhi, P., *Mol. Breeding*, 2000, **6**, 489–499.
54. Nakayama, H., Yoshida, K., Ono, H., Murooka, Y. and Shinmyo, A., *Plant Physiol.*, 2000, **122**, 1239–1247.
55. Xu, D., Duan, X., Wang, B., Hong, B., David Ho, T. H. and Wu, R., *ibid*, 1996, **110,** 249–257.
56. Sheveleva, E., Chmara, W., Bohnert, H. J. and Jensen, R. G., *ibid*, 1997, **115**, 1211–1219.
57. Vernon, D. M., Tarczynski, M. C., Jensen, R. G. and Bohnert, H. J., *Plant J.*, 1993, **4**, 199–205.
58. Tarczynski, M. C., Jensen, R. G. and Bohnert, H. J., *Science*, 1993, **259**, 508–510.
59. Traczynski, M. C., Jensen, R. G. and Bohnert, H. J., *ibid*, 1992, **89**, 2600–2604.
60. Thomas, J. C., Sepahi, M., Arendall, B. and Bohnert, H. J., *Plant Cell Environ.*, 1995, **18**, 801–806.
61. Shen, B., Jensen, R. G. and Bonhert, H. J., *Plant Physiol.*, 1997, **113**, 1177–1183.
62. Pilon-Smits, E. A. H., *J. Plant Physiol.*, 1998, **152**, 525–532.
63. Kishor, K. P. K., Hong, Z., Miao, G. H., Hu, C. A. A. and Verma, D. P. S., *Plant Physiol.*, 1995, **108**, 1387–1394.
64. Hong, Z., Lakkineni, K., Zhang, Z. and Verma, D. P. S., *ibid*, 2000, **122**, 1129–1136.
65. Zhu, B., Su, J., Chang, M., Verma, D. P. S., Fan, Y. L. and Wu, R., *Plant Sci.*, 1998, **199**, 41–48.
66. Nanjo, T., Kobayashi, M., Yoshiba, Y., Kakubari, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K., *FEBS Lett.*, 1999, **461**, 205–210.
67. Pilon-Smits, E. A. H., Ebskamp, M. J. M., Paul, M. J., Jeuken, M. J. W., Weisbeek, P. J. and Smeekens, S. C. M., *Plant Physiol.*, 1995, **107**, 125–130.
68. Holmstrom, K. O., Mantyl, E., Welin, B., Mandal, A. and Palva, E. T., *Nature*, 1996, **379**, 683–684.
69. Romero, C., Belles, J. M., Vaya, J. L., Serrano, R. and Culianez-Macia, F. A., *Planta*, 1997, **201**, 293–297.
70. Gomes, E., Jakobsen, M. K., Axelsen, K. B., Geisler, M. and Palmgram, M. G., *Plant Cell*, 2000, **12**, 2441–2453.
71. Apse, M. P., Aharon, G. S., Snedden, W. A. and Blumwald, E., *Science*, 1999, **285**, 1256–1258.
72. Zhang, H. X. and Blumwald, E., *Nature Biotechnol.*, 2001, **19**, 765–768.
73. Zhang, H. X., Hudson, N. J., Williams, J. N. and Blumwald, E., *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 12832–12836.
74. Gisbert, C. *et al.*, *Plant Physiol.*, 2000, **123**, 393–402.
75. Hightower, R., Banden, C., Penzes, E., Lund, P. and Dunsmuir, P., *Plant Mol. Biol.*, 1991, **17**, 1013–1021.
76. Wallis, J. G., Wang, H. and Guerra, D. J., *ibid*, 1997, **35**, 323–330.
77. Iuchi, S. *et al.*, *Plant J.*, 2001, **27**, 325–333.
78. Alvim, F. C., Carolino, S. M. B., Cascardo, J. C. M., Nunes, C. C., Martinez, C. A., Otoni, W. C. and Fontes, E. P. B., *Plant Physiol.*, 2001, **26**, 1042–1054.
79. Artus, N. N., Uemura,. M., Stepnokus, P. L., Gilmour, S. J., Lin, C. and Thomashow, M. F., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 13404–13409.
80. Veena, Reddy, V. S. and Sopory, S. K., *Plant J.*, 1999, **17**, 385–395.
81. Jeong, M. J., Park, S. C. and Byun, M. O., *Mol. Cells*, 2001, **12**, 185–189.
82. Hoshida, H., Tanaka, Y., Hibino, T., Hayashi, Y., Tanaka, A., Takabe, T. and Takabe, T., *Plant Mol. Biol.*, 2000, **43**, 103–111.
83. Grover, A., Pareek, A. and Maheshwari, S. C., *Proc. Indian Natl. Acad. Sci.*, 1993, **B59**, 113–127.
84. Grover, A., Hossain, M. A., Huq, M. E., McGee, J. D., Peacock, W. J., Dennis, E. S. and Hodges, T. K., Proceedings of the International Rice Research Conference on Fragile lives in fragile ecosystems, Manila, Philippines, 1995, pp. 911–921.
85. Singla, S. L., Pareek, A. and Grover, A., in *Plant Ecophysiology* (ed. Prasad M. N. V.), John Wiley, New York, 1997, pp. 101–127.
86. Pareek, A., Singla, S. L. and Grover, A., in *Strategies for Improving Salt Tolerance in Higher Plants* (eds Jaiswal, P. K., Singh, R. P. and Gulati, A.), Oxford & IBH, New Delhi, 1997, pp. 365–391.
87. Grover, A., Sanan, N. and Sahi, C., *Curr. Sci.*, 1998, **75**, 178–179.
88. Grover, A. *et al.*, *ibid*, 1998, **75,** 689–696.
89. Grover, A., *ibid*, 1999, **76,** 136–137.
90. Grover, A., Sahi, C., Sanan, N. and Grover, A., *Plant Sci.*, 1999, **143**, 101–111.
91. Minhas, D. and Grover, A., *Plant Sci.*, 1999, **146,** 41–51.
92. Katiyar-Agarwal, S., Agarwal, M. and Grover, A., *Curr. Sci.*, 1999, **77**, 1577–1579.
93. Grover, A. and Minhas. D., *Proc. Indian Natl. Acad. Sci.*, 2000, **B66**, 13–32.
94. Grover, A., in *Probing Photosynthesis: Mechanism, Regulation and Adaptation* (eds Yunus, M., Pathre, U. and Mohanty, P.), Taylor and Francis, London, 2000, pp. 397–408.
95. Grover, A. *et al.*, *Curr. Sci.*, 2001, **80**, 206–216.
96. Grover, A. *et al.*, *Proc. Indian Natl. Acad. Sci.*, 2001, **B67**, 189–214.
97. Katiyar-Agarwal, S., Agarwal, M., Gallie, D. R. and Grover, A., *Crit. Rev. Plant Sci.*, 2001, **20**, 277–295.
98. Agarwal, M., Katiyar-Agarwal, S., Sahi, C., Gallie, D. and Grover, A., *Cell Stress Chaperones*, 2001, **6**, 219–224.
99. Grover, A., *ibid*, 2002, **7**, 1–5.
100. Grover, A. and Chandramouli, A., *Physiol. Mol. Biol. Plants*, 2002, **8**, 193–211.
101. Shibata, D. and Liu, Y-G., *Trends Plant Sci.*, 2000, **5,** 354–357.
102. Reddy, M. and Gowrishankar, J., *J. Bacteriol.*, 2000, **182**, 1978–1986.
103. Sai Sree, L., Reddy, M. and Gowrishankar, J., *J. Bacteriol.*, 2000, **182**, 3151–3157.
104. UmaPrasad, G. and Gowrishankar, J., *J. Genet.*, 1998, **77**, 1–11.
105. Apte, S. K. and Haselkorn, R., *Plant Mol. Biol.*, 1990, **15**, 723–733.
106. Apte, S. K., Fernandes, T. A., Iyer, V. and Alahari, A., in *Plant Molecular Biology and Biotechnology* (eds Tewari, K. K. and Singhal, G. S.), Narosa Publishing House, New Delhi, pp. 258–268.
107. Lakhotia, S. C., *J. Genet.*, 1987, **66**, 139–157.
108. Lakhotia, S. C., Ray, P., Rajendra, T. K. and Prasanth, K. V., *Curr. Sci.*, 1999, **77**, 553–563.
109. Sinha, S. K. and Khanna, R., *Adv. Agron.*, 1975, **27**, 123–174.
110. Khanna-Chopra, R. and Sinha, S. K., *Curr. Sci.*, 1998, **74**, 25–34.
111. Khanna-Chopra, R. and Maheshwari, M., *Eur. J. Agron.*, 1998, **9**, 101–107.
112. Khanna-Chopra, R., Dalal, M., Pradeep Kumar, G. and Laloraya, M., *Plant Physiol.*, 1998, **117**, 9–18.
113. Uma, S., Prasad, T. G. and Udaya Kumar, M., *Ann. Bot.*, 1995, **76**, 43–49.
114. Jayaprakash, T. L., Ramamohan, G., Krishna Prasad, B. T., Ganesh Kumar, Prasad, T. G., Mathew, M. K. and Udaya Kumar, M., *ibid*, 1998, **82**, 513–522.
115. Kumar, G., Krishinaprasad, B. T., Saritha, M., Gopalakrishna, R., Mukhopadhyay, K., Ramamohan, G. and Udaya Kumar, M., *Theor. Appl. Genet.*, 1999, **99**, 359–367.
116. Hemamalini, G. S., Shashidhar, H. E. and Hittalmani, S., *Euphytica*, 2000, **112,** 69–78.

117. Deswal, R., Pandey, G. P., Chandok, M. R., Yadav, N., Bhattacharya, A. and Sopory, S. K., *Eur. J. Biochem.*, 2000, **267**, 3181–3188.
118. Pandey, S., Tiwari, S. B., Upadhyaya, K. C. and Sopory, S. K., *Crit. Rev. Plant Sci.*, 2000, **19**, 291–310.
119. Kumria, R., Waie, B. and Rajam, M. V., *Plant Cell Tissue Org. Cult.*, 2001, **67**, 63–71.
120. Shoeb, F., Yadav, J. S., Bajaj, S. and Rajam, M. V., *Plant Sci.*, 2001, **160**, 1229–1235.
121. Kumar, S. V., Yadav, J. S., Verma, R. and Rajam, M. V., *Plant Cell Rep.*, 2001, communicated.
122. Sharma, P. and Rajam, M. V., *J. Exp. Bot.*, 1995, **46,** 135–141.
123. Yadav, J. S. and Rajam, M. V., *Plant Physiol.*, 1998, **116,** 617–625.
124. Kumria, R. and Rajam, M. V., *J. Plant Physiol.*, 2002, **159**, 983–990.
125. Prabhavati, V., Yadav, J. S., Kumar, P. A. and Rajam, M. V., *Mol. Breed.*, 2002 (in press).
126. Prasad, K. V. S. K., Sharmila, P. and Pardha Saradhi, P., *Plant Sci.*, 2000 (in press).
127. Mohanty, A., Kathuria, H., Ferjani, A., Sakamoto, A., Murata, N., Mohanty, P. and Tyagi, A. K., *Theor. Appl. Genet.*, 2002, **106**, 51–57.
128. Singla, S. L. and Grover, A., *Plant Mol. Biol.*, 1993, **22**, 1177–1180.
129. Pareek, A., Singla, S. L. and Grover, A., *Plant Mol. Biol.*, 1995, **29**, 293–301.
130. Singla, S. L., Pareek, A. and Grover, A., *ibid.*, 1998, **37**, 911–919.
131. Agarwal, M. *et al.*, *ibid*, 2003 (in press).
132. Grover, A. *et al.*, in Proceedings of the Third International Rice Genetics Symposium, IRRI, Manila, Philippines, 2000 (in press).
133. Bansal, K. C. *et al.*, in *Biotechnology in Horticultural and Plantation Crops* (eds Chadha, K. L., Ravindran, P. N. and Sahijram, L.), Malhotra Publishing, New Delhi, 2000 pp. 300–312.
134. Barthakur, S., Babu, V. and Bansal, K. C., *Plant Biochem. Biotech.*, 2001, **10**, 31–37.
135. Sahi, C., Agarwal, M., Reddy, M. K., Sopory, S. K. and Grover, A., *Theor. Appl. Genet.*, 2003 (in press).
136. Mukopadhyay, A., Sharma, S. and Tyagi, A., *Plant Biochem. Biotechnol.*, 2001, **10**, 25–30.
137. Pareek, A., Singla, S. L. and Grover, A., *Curr. Sci.*, 1998, **75**, 1023–1035.
138. Pareek, A., Singla, S. L. and Grover, A., *ibid*, 1998, **75**, 1170–1174.
139. Pareek, A., Singla, S. L. and Grover, A., *ibid*, 1999, **76**, 81–86.
140. Minhas, D. and Grover, A., *Plant Sci.*, 1999, **146**, 41–51.
141. Dubey, H. and Grover, A., *ibid*, 2003 (in press).
142. Rao, A. H., Karunasree, B. and Reddy, A. R., *J. Plant Physiol.*, 1993, **142**, 88–93.
143. Karuna Sree, B., Rajendrakumar, C. S. and Reddy, A. R., *Plant Sci.*, 2000, **160**, 149–157.
144. Rajendrakumar, C. S., Suryanarayana, T. and Reddy, A. R., *FEBS Lett.*, 1997, **410**, 201–205.
145. Ramani, S. and Apte, S. K., *Biochem. Biophys. Res. Commun.*, 1997, **233**, 663–667.
146. Ghosh, S., Bagchi, S. and Majumdar, A. L., *Plant Sci.*, 2001, **160**, 1171–1181.
147. Gupta, S., Chattopahdyay, M. K., Chatterjee, P., Ghosh, B. and Sengupta, D. N., *Plant Mol. Biol.*, 1998, **37**, 629–637.
148. Sengupta, D., Banerjee, K. and Rouf, A., *J. Plant Biol.*, 1999, **26**, 176–185.

ACKNOWLEDGEMENTS. A.G. thanks the Department of Biotechnology, Government of India (BT/AB/05/02/91-Vol. IV) and National Agricultural Technology Project (28(1)/99-NATP/CGP-I/33) for financial support to his laboratory. S.K.-A. and M.A. thank Council of Scientific and Industrial Research (CSIR) for the fellowship awards.

The current status of plant transformation technologies

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Plant transformation vectors and methodologies have been improved to increase the efficiency of plant transformation and to achieve stable expression of transgenes in plants. Due to the simplicity of the transformation system and precise integration of transgenes, *Agrobacterium* Ti plasmid-based vectors continue to offer the best system for plant transformation. Binary vectors have been improved by the incorporation of supervirulent *vir* genes, matrix attachment regions (MAR) and the insertion of introns in marker genes and reporter genes. With these improvements and with the use of acetosyringone, transformation of monocotyledonous plants using *Agrobacterium* has almost become a routine process. The green fluorescent protein (GFP) gene has been extensively modified for plant codon preference, ER/plastid targeting and for greater solubility thereby making it a versatile vital reporter for transgenic plants. Significant progress is seen in developing transgenic plants devoid of antibiotic marker genes. Cotransformation of multiple T-DNAs, site-specific recombination strategies and deployment of Ac/Ds-based transposition have helped in the elimination of marker genes in transgenic plants. Positive selection strategies using *ipt*, xylose isomerase and phospho-mannose isomerase have been demonstrated to be useful in many crop plants. The development of BIBAC vectors, a demonstrated capability to transfer multiple genes of a pathway and successful T-DNA tagging in rice, signal the readiness with which transformation technologies can be deployed for the study of 'functional genomics' in plants. The particle bombardment system continues to find use in organelle transformation and transformation of plants that lack efficient regeneration systems. A detailed understanding of gene silencing has led to the design of vectors that minimize transgene silencing while ensuring desired levels of transgene expression. Efforts are underway to understand the mechanism of T-DNA integration in plants so that 'knock out' mutagenesis and homology-based gene replacements can be achieved in plants. We review in this article the current status of transformation technologies. An overview of the status of deployment of plant transformation technologies in India is also presented.

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Plant transformation is performed using a wide range of tools such as *Agrobacterium* Ti plasmid vectors, microprojectile bombardment, microinjection, chemical (PEG) treatment of protoplasts and electroporation of protoplasts. Though all methods have advantages that are unique to each of them, transformation using *Agrobacterium* and microprojectile bombardment are currently the most extensively used methods[1]. Recent developments in these two technologies have been reviewed together with the phenomenon of 'gene silencing' that has come to centrestage after a large number of transgenic plants have been carefully evaluated for transgene expression in successive generations.

Agrobacterium-mediated gene transfer

The naturally evolved unique ability of *Agrobacterium tumefaciens* to precisely transfer defined DNA sequences to plant cells has been very effectively utilized in the design of a range of Ti plasmid-based vectors. The current status of our understanding of *Agrobacterium* T-DNA transfer process has been reviewed by Gelvin[2] and Zupan *et al.*[3]. Three genetic elements, *Agrobacterium* chromosomal virulence genes (*chv*), T-DNA delimited by a right border and a left border and Ti plasmid virulence genes (*vir*) constitute the T-DNA transfer machinery. Important events of T-DNA transfer and components involved in the process are outlined in Figure 1. The mechanisms governing the transfer of 'T-complex' via the conjugation channel and the roles of plant and *Agrobacterium* proteins in T-DNA integration are being intensely studied.

Agrobacterium-based DNA transfer system offers many unique advantages in plant transformation: (1) The simplicity of *Agrobacterium* gene transfer makes it a 'poor man's vector. (2) A precise transfer and integration of DNA sequences with defined ends. (3) A linked transfer of genes of interest along with the transformation marker. (4) The higher frequency of stable transformation with many single copy insertions. (5) Reasonably low incidence of transgene silencing. (6) The ability to transfer long stretches of T-DNA (> 150 kb).

For long, the inability of *Agrobacterium* to transfer DNA to monocotyledonous plants was considered its major limitation. However, with effective modifications