Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes
Tony H H Chen* and Norio Murata†

The accumulation of compatible solutes, such as betaines, proline and sugar alcohols, is a widespread response that may protect plants against environmental stress. It is not yet fully understood how these compounds are involved in the stress tolerance of whole plants. Some plants have been genetically engineered to express enzymes that catalyze the synthesis of various compatible solutes. Some interventions have increased the tolerance of some crop plants to abiotic stress. Furthermore, analysis of such transgenic plants has begun to clarify the roles of compatible solutes in stress tolerance.

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Abbreviations
BADH betainealdehyde dehydrogenase
CaMV cauliflower mosaic virus
CDH choline dehydrogenase
CMO choline monoxygenase
CodA choline oxidase
COR C O ld Regulated
dw dry weight
fw fresh weight
GB glycine betaine
MDA malondialdehyde
mt1D mannitol-1-phosphate dehydrogenase gene
P5CS Δ1-pyrroline-5-carboxylate synthase
PEAMT phosphoethanolamine N-methyltransferase
ROS reactive oxygen species
S6PDH sorbitol-6-phosphate dehydrogenase
TPS trehalose-6-phosphate synthase

Introduction
In the natural environment, plants often grow under unfavorable conditions, such as drought, salinity, chilling, freezing, high temperature, flooding, or strong light. These conditions are known collectively as abiotic stresses, and any of them can delay growth and development, reduce productivity and, in extreme cases, cause the plant to die. To ensure their own survival and the prosperity of their offspring, plants have evolved a range of strategies to cope with various abiotic stresses. One common mechanism is the accumulation of compatible solutes, that is, low-molecular-weight, highly soluble compounds that are nontoxic at high concentrations. The compatible solutes that are accumulated differ among plant species and can include betaines and related compounds; polyols and sugars, such as mannitol, sorbitol, and trehalose; and amino acids, such as proline [1,2].

Genetic transformation has allowed the introduction of new pathways for the biosynthesis of various compatible solutes into plants, resulting in the production of transgenic plants with improved tolerance to stress [2]. Considerable progress has been made in engineering the biosynthesis of compatible solutes in a variety of species, including some agriculturally important crops. In this review, we summarize information about the roles of compatible solutes in stress tolerance that has been made available through analyses of transgenic plants.

Transgenic plants engineered to synthesize glycine betaine for enhanced tolerance to stress

Betaines are quaternary ammonium compounds in which the nitrogen atom is fully methylated. The most common betaines in plants include glycine betaine (GB; the most widely studied betaine), as well as proline betaine, β-alanine betaine, choline-O-sulfate and 3-dimethylsulfo-niopropionate [1,2]. Glycine betaine is widely distributed in higher plants and is synthesized in many plant species at elevated rates in response to various types of environmental stress [1]. Whereas several taxonomically distant species are accumulators of GB, others, such as Arabidopsis, rice (Oryza sativa), and tobacco (Nicotiana tabacum) are considered to be non-accumulators [1,2].

Glycine betaine appears to be a critical determinant of stress tolerance. Its accumulation is induced under stress conditions, with its concentration being correlated with the level of tolerance [1]. Moreover, exogenous application of GB improves the growth and survival of a wide variety of plants under various stresses (for example, see [3,4,5*]). In addition, studies in vitro have shown that GB is effective as a compatible solute in stabilizing the quaternary structures of enzymes and complex proteins, as well as in maintaining the highly ordered state of membranes, at high concentrations of salts or at extreme temperatures [6]. Finally, the introduction of a pathway for the biosynthesis of GB into non-accumulators of GB has proved to be effective in increasing their tolerance of various abiotic stresses (for reviews, see [2,7**,9**]).

Glycine betaine is synthesized from either choline or glycine, via two distinct pathways: dehydrogenation of choline or N-methylation of glycine (Figure 1). For almost
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all biological systems, including most animals, plants, and microorganisms, GB synthesis is accomplished by conversion of choline to GB through a two-step dehydrogenation/oxygenation via the unstable intermediate betaine aldehyde. In *E. coli*, two enzymes, choline dehydrogenase (CDH) and betaine aldehyde dehydrogenase (BADH), are required [10]. In higher plants, GB is synthesized in chloroplasts from choline by choline monooxygenase (CMO) and BADH [11]. In contrast, GB synthesis in the microorganisms *Arthrobacter globiformis* and a closely related strain *Arthrobacter pascens* requires only one enzyme choline oxidase (i.e. codA from *Arthrobacter globiformis* [15] and cox from *Arthrobacter pascens* [16]); and glycine sarcosine methyltransferase (GSMT) and sarcosine dimethylglycine methyltransferase (SDMT) from both *Actinopolyspora halophilia* and *Ectothiorhodospira halochloris* [13••]. Transgenic plants of various species (Table 1) have been produced that express all of these genes individually, with the exception of the last two. These plants accumulate GB at various levels and exhibit enhanced tolerance of several types of stress [7••–9••].

Genes that encode the enzymes involved in the biosynthesis of GB have been cloned. They include genes for choline monooxygenase (CMO) and BADH from higher plants [11]; CDH and BADH from *Escherichia coli* [14]; choline oxidase (i.e. codA from *Arthrobacter globiformis* [15] and cox from *Arthrobacter pascens* [16]); and glycine sarcosine methyltransferase (GSMT) and sarcosine dimethylglycine methyltransferase (SDMT) from both *Actinopolyspora halophilia* and *Ectothiorhodospira halochloris* [13••].

Choline-dehydrogenation/oxidation pathways

(a) Plants

\[
\begin{align*}
\text{Choline} & \xrightarrow{\text{CMO}} \text{Betaine aldehyde} \\
\text{H}_3\text{C}-\text{N}^+\text{CH}_3 & \xrightarrow{2\text{Fd(red)}} \text{H}_3\text{C}-\text{N}^+\text{CH}_3 \\
\text{BADH} & \text{Glycine betaine} \\
\end{align*}
\]

(b) *Escherichia coli*

\[
\begin{align*}
\text{Choline} & \xrightarrow{\text{CDH}} \text{Betaine aldehyde} \\
\text{H}_3\text{C}-\text{N}^+\text{CH}_3 & \xrightarrow{\text{BADH}} \text{Glycine betaine} \\
\end{align*}
\]

(c) *Arthrobacter globiformis*

\[
\begin{align*}
\text{Choline} & \xrightarrow{\text{COD}} \text{Betaine aldehyde} \\
\text{H}_3\text{C}-\text{N}^+\text{CH}_3 & \xrightarrow{2\text{H}_2\text{O} + 2\text{H}_2\text{O}_2} \text{Glycine betaine} \\
\end{align*}
\]

Glycine methylation pathway

\[
\begin{align*}
\text{Glycine} & \xrightarrow{\text{GSMT}} \text{Sarcosine (N-methylglycine)} \\
\text{H}_3\text{C}-\text{N}^+\text{H} & \xrightarrow{\text{SDMT}} \text{N, N-Dimethyl glycine} \\
\text{Glycine betaine} & \\
\end{align*}
\]

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Figure 1

Pathways for the biosynthesis of glycine betaine. COD, choline oxidase; GSMT, glycine sarcosine methyltransferase; SDMT, sarcosine dimethyltransferase; Fd(red) and Fd(ox), ferredoxin in reduced and oxidized forms, respectively; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.
The metabolic engineering of plants to endow them with the ability to synthesize GB might appear to be an effective method for improving stress tolerance [2,7••–9••]. However, the observed concentrations of GB in such transgenic plants are generally low (<5 µmol g⁻¹ fresh weight [fw]) compared with the levels observed in stressed plants of species that normally accumulate GB when under stress (4–40 µmol g⁻¹ fw [1]). This has proven to be the case for plants engineered to express the bet gene of E. coli, which encodes CDH and BADH (with or without modified codon usage) [10]; plant genes for CMO and BADH [11,17]; or the cox [18•] and codA genes [19] — regardless of the species of host plant [8••]. Therefore, it seems that choline-oxidizing enzymes might not be the factor that limits the accumulation of GB. Indeed, two major factors have been identified that limit accumulation of GB in transgenic plants: the availability of endogenous choline itself [2,14,17,18•] and the transport of choline across the chloroplast envelope [20•,21].

Tobacco plants that expressed a spinach gene for CMO in their chloroplasts had low levels of CMO activity and produced little GB (≤70 nmol g⁻¹ fw) [20•]. Moreover, variations in CMO activity of up to 100-fold among individual transformants did not produce any significant effects on the GB concentration [20•]. When CMO was expressed in the cytosol, however, transgenic tobacco plants did accumulate more GB (430 nmol g⁻¹ fw), about five-fold more than in transgenic plants that accumulated GB in their chloroplasts. Modeling of the kinetics of labeled choline metabolites, after the application of [14C]-choline to transgenic plants, demonstrated that choline import into the chloroplasts was a major constraint on the synthesis of GB in these organelles [21].

The key enzyme in the choline-biosynthetic pathway is phosphoethanolamine N-methyltransferase (PEAMT; EC 2.1.1.103), which catalyzes each of the three methylation reactions that are required to convert phosphoethanolamine to phosphocholine [22••]. A gene for PEAMT has been isolated from spinach and used to transform tobacco plants that coexpressed both spinach CMO and beet BADH; thus, these transformants were able to synthesize GB in their chloroplasts [22••]. The transgenic plants that expressed PEAMT contained up to 50-fold more free choline and accumulated 30-fold more GB than plants transformed with the vector alone. The highest level of GB produced was 1.8 µmol g⁻¹ fw in greenhouse-grown plants, which was about 45-fold higher than the concentration in plants that had been transformed with the vector alone. Despite the simultaneous expression of CMO, BADH and PEAMT, the level of GB that accumulated in transgenic plants was still lower than the 5 µmol g⁻¹ fw reported for transgenic rice plants that expressed either a modified betA gene in their mitochondria [10] or a codA gene in their cytosol [23].

### Transgenic plants engineered to produce compatible solutes other than glycine betaine

In addition to plants that have been engineered to synthesize GB, transgenic plants that accumulate various other compatible solutes have also been produced (Table 2).

### Fructan

To assess the effects of fructan on the abiotic stress tolerance of plants, tobacco was transformed with a construct that contained the sacB gene for levansucrase from Bacillus subtilis fused to the vacuole-sorting signal of carboxypeptidase Y from yeast, placed downstream of the constitutive 35S promoter of cauliflower mosaic virus (CaMV) [24]. Levansucrase generates fructan from fructose. The fructan-producing plants performed significantly better than controls under drought conditions, having a 55% more rapid growth rate, 33% greater fresh weight and 59% greater dry weight than wildtype plants. Under drought conditions, the transgenic plants accumulated fructan to concentrations as high as 0.35 mg g⁻¹ fw, close to 7-fold higher than the level accumulated by the same plants

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**Table 1**

<table>
<thead>
<tr>
<th>Species</th>
<th>Gene</th>
<th>Maximal accumulation</th>
<th>Enhanced tolerance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabidopsis thaliana</td>
<td>codA</td>
<td>1.2 µmol g⁻¹ fw</td>
<td>Chilling</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>codA</td>
<td>1.2 µmol g⁻¹ fw</td>
<td>Chilling, salt</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>codA</td>
<td>1.2 µmol g⁻¹ fw</td>
<td>Heat</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td>codA</td>
<td>1.2 µmol g⁻¹ fw</td>
<td>Strong light</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>codA</td>
<td>1.2 µmol g⁻¹ fw</td>
<td>Salt</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td>codA</td>
<td>1.2 µmol g⁻¹ fw</td>
<td>Freezing</td>
<td>[47•]</td>
</tr>
<tr>
<td></td>
<td>codA</td>
<td>19 µmol g⁻¹ dw</td>
<td>Freezing, salt</td>
<td>[18•]</td>
</tr>
<tr>
<td>Brassica napus</td>
<td>cox</td>
<td>13 µmol g⁻¹ dw</td>
<td>Drought, salt</td>
<td>[18•]</td>
</tr>
<tr>
<td>Brassica juncea</td>
<td>codA</td>
<td>0.82 µmol g⁻¹ fw</td>
<td>Salt</td>
<td>[48]</td>
</tr>
<tr>
<td>Diospyros kaki</td>
<td>cox</td>
<td>0.3 µmol g⁻¹ fw</td>
<td>Salt</td>
<td>[48]</td>
</tr>
<tr>
<td>Nicotiana tabacum</td>
<td>betA</td>
<td>?*</td>
<td>Salt</td>
<td>[18•]</td>
</tr>
<tr>
<td></td>
<td>betA/betB</td>
<td>0.035 µmol g⁻¹ fw</td>
<td>Chilling, salt</td>
<td>[51]</td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>codA</td>
<td>5.3 µmol g⁻¹ fw</td>
<td>Chilling, salt</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>betA (modified)</td>
<td>5.0 µmol g⁻¹ fw</td>
<td>Drought, salt</td>
<td>[10]</td>
</tr>
</tbody>
</table>

*No data were given.*
under non-stress conditions. The same construct was also inserted into sugar beet (Beta vulgaris L.) [25]. The resulting transgenic beet plants accumulated fructan to about 0.5% of their dry weight in both root and shoots. Moreover, these beet transgenics grew significantly better under drought conditions than did wildtype plants.

**Mannitol**

Tobacco and Arabidopsis plants do not usually contain mannitol. However, expression of the *mt1D* gene for mannitol-1-phosphate dehydrogenase from *E. coli* in these two species resulted in the biosynthesis of mannitol. Thus, for example, mannitol accumulated to more than 6 µmol g⁻¹ fw in the leaves of some transgenic tobacco plants [26] and to close to 3 µmol g⁻¹ fw in the leaves of transgenic Arabidopsis [27]. The mannitol-producing tobacco plants exhibited increased tolerance of high salinity [28]. However, the mannitol-accumulating transgenic tobacco plants were 20–25% smaller than wildtype plants under non-stressed conditions [29]. Salt stress (i.e. 150 mM NaCl) reduced the dry weight of wildtype plants by 44% but had no effect on the dry weight of the transgenic plants. Moreover, the transgenic plants adjusted to osmotic stress, whereas wildtype plants did not. Shen et al. [30] transformed tobacco with a construct in which the *mt1D* enzyme was targeted to chloroplasts. The resulting transgenic plants accumulated mannitol at concentrations from 2.5 to 7.0 µmol g⁻¹ fw. The presence of mannitol in the chloroplasts resulted in enhanced resistance to oxidative stress induced by the presence of methyl viologen. Such resistance was due to an increased capacity to scavenge hydroxyl radicals. In the seeds of mannitol-accumulating Arabidopsis plants, the concentration of mannitol reached 10 µmol g⁻¹ dry weight (dw) [27]. Mannitol-expressing seeds were able to germinate in medium supplemented with up to 400 mM NaCl, whereas control seeds ceased to germinate at 100 mM NaCl.

**D-O-nonitol**

Sheveleva et al. [31] generated transgenic tobacco plants that expressed a cDNA that encoded D-myo-inositol O-methyltransferase from the ice plant Mesembryanthemum crystallinum. When these transgenic plants were exposed to salt or drought stress, D-o-nonitol (a sugar alcohol) accumulated to concentrations that exceeded 35 µmol g⁻¹ fw in the cytosol. Furthermore, the photosynthetic fixation of CO₂ was inhibited to a lesser extent during salt or drought stress in the transgenic plants that accumulated D-o-nonitol than in wildtype plants.

**Proline**

Δ¹-Pyrroline-5-carboxylate synthase (P5CS) is subject to feedback inhibition by proline. Therefore, Hong et al. [32*] used site-directed mutagenesis to replace the Phe residue at position 129 in P5CS from *V. aconitifolia* with an Ala residue. The mutated enzyme (P5CSF129A) was no longer subject to feedback inhibition. Plants that expressed P5CSF129A accumulated about twice as much proline as those that expressed the wildtype P5CS. The elevated level of proline significantly enhanced the ability of the transgenic seedlings to grow in medium that contained up to 200 mM NaCl. The increased level of proline also reduced the levels of free radicals, as determined by monitoring the production of malondialdehyde (MDA). Thus, it appears that, in addition to acting as an osmolyte, proline might play a role in reducing the oxidative stress that is brought on by osmotic stress. Nanjo et al. [33] generated transgenic Arabidopsis plants using AtProDH cDNA, which encodes the antisense gene for proline dehydrogenase, an enzyme that catalyzes the degradation
of proline. These transgenic plants accumulated higher levels of proline than did wildtype plants. They also exhibited increased tolerance of both freezing stress (–7°C for two days) and salt stress (600 mM of NaCl).

**Sorbitol**
Sheveleva et al. [34] produced and analyzed transgenic tobacco lines that expressed a cDNA for sorbitol-6-phosphate dehydrogenase (S6PDH) from apple. The amount of sorbitol produced by these transgenics varied from 0.2 to 130 µmol g⁻¹ fw. Plants that accumulated up to 2–3 µmol g⁻¹ fw sorbitol were phenotypically normal, but plants that accumulated higher levels developed necrotic lesions on their leaves, infertility, and/or the inability to regenerate roots. An S6PDH construct was also used to transform Japanese persimmon [35], and the levels of sorbitol detected in the transgenic plants ranged from 14.5–61.5 µmol g⁻¹ fw. Under salt stress, photosynthetic activity declined less in the leaves of sorbitol-producing Japanese persimmon than in the leaves of wildtype plants, indicating an increase in the ability of sorbitol-producing plants to tolerate salt stress.

**Trehalose**
Holmström et al. [36] transformed tobacco with the gene for the trehalose-6-phosphate synthase (TPS1) subunit of yeast trehalose synthase, which was driven by the promoter of the rbcS gene from Arabidopsis. The TPS1-positive plants contained 0.8–3.2 mg g⁻¹ dw trehalose but exhibited a 30–50% reduction in growth rate compared to controls. The accumulation of trehalose seemed to improve drought tolerance. Expression of the yeast gene for TPS1 in tobacco, driven by the drought-inducible promoter of RD29 (RESPONSIVE TO DESSICATION29) [37], also improved the drought tolerance of the resultant trehalose-accumulating plants. Some side effects of trehalose accumulation were observed, however, including stunted growth, the production of lancet-shaped leaves, and a reduction in sucrose levels. The yeast gene for TPS1, driven by the 35S promoter of CaMV, has been used to transform potato (Solanum tuberosum) [38]. The transgenic potato plants exhibited significantly enhanced tolerance to drought. However, as in the case of the transgenic tobacco plants that expressed TPS1, the potato transgenics exhibited various morphological changes, which ranged from severely retarded growth to yellowish, lancet-shaped leaves and the aberrant development of roots.

Other genes for the synthesis of trehalose have been used to generate trehalose-accumulating plants. Transgenic tobacco plants have been produced that expressed the *otsA* and *otsB* genes from *E. coli*, which encode trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase, respectively [39]. Very low levels of trehalose (8 µg g⁻¹ fw and 90 µg g⁻¹ fw in the case of expression of *otsA* and *otsB*, respectively) were detected in the leaves of the transformed plants. Nonetheless, the transgenic plants were more drought-tolerant than controls, even though they exhibited substantial changes in morphology and accumulated higher levels of non-structural carbohydrates.

**Mechanisms of protection against the damaging effects of stress**
With the exception of the above-described transgenic plants that accumulated proline and D-ononitol, respectively, most of the transgenic plants that we have discussed contained only low levels of the specific products of interest, and these concentrations were insufficient to be of osmotic importance. Nevertheless, moderate to high levels of stress tolerance were reported for these transgenics. Therefore, protective mechanisms other than direct osmoprotection were probably responsible for the increased tolerance of stress. Possible roles for GB in stress tolerance include stabilization by GB of complex proteins and membranes *in vivo*, protection by GB of the transcriptional and translational machinery, and intervention by GB as a molecular chaperone in the refolding of enzymes [8**]. In addition, GB might reduce the peroxidation of membrane lipids [5*] and might protect electron transport via complex II in mitochondria [40].

The role of reactive oxygen species (ROS) in inducing damage to plants under stress conditions and the role of compatible solutes in the protection of plants against stress have been discussed by many authors [41]. It seems likely that ROS, which are synthesized by plants experiencing various stress conditions, directly damage cellular components, such as membrane lipids [41] and the photosystem II complex [41]. It also seems likely that ROS are scavenged by compatible solutes, resulting in the protection of plants against stress conditions. Furthermore, *in vitro* experiments, some compatible solutes — such as mannitol, proline and sorbitol, but not GB — have been shown to be effective scavengers of ROS [42]. Transgenic plants that synthesize compatible solutes, with the exception of GB, exhibit enhanced tolerance to oxidative stress [30,32*]. However, the direct quantification of ROS *in vivo* is difficult, and most researchers have used the production of MDA as an indicator of the production of ROS. This method does not provide an accurate assessment of the true concentrations of ROS in plant cells. Moreover, the threshold concentrations of ROS that induce direct damage to cellular components are unknown. Thus, the significance of the peroxidation of lipids by ROS *in vivo* and its relationship to actual cellular damage remain to be clarified. There is not enough evidence to support current opinion about the possible roles of ROS in causing stress-induced damage and how such damage might be prevented.

There is, however, a plausible scheme that might better explain the results described above. The fates of cellular components under stress conditions depend on the balance between rate of damage and rate of repair. When the rate of repair of a given component is more rapid than the rate of damage, no damage becomes apparent. This situation is probably the norm in plants under non-stress conditions.
As the level of stress increases, the balance tips gradually toward damage. When the rate of repair is slower than the rate of damage, damage becomes apparent. This is probably the situation in plants under stress. Such a relationship between damage and repair has been clearly demonstrated in the photoinhibition of photosystem II complexes [43••]. Therefore, the involvement of ROS in the damage that occurs under stress conditions and of protection by compatible solutes can be explained as follows. Various kinds of stress increase levels of ROS, and the elevated levels of ROS might inhibit repair processes, which are linked (for the most part) to protein synthesis. In cyanobacterial cells, a model system for studies of chloroplasts, H₂O₂ inhibits protein synthesis, which is essential for the repair of the photodamaged photosystem II complex, even when the concentration of H₂O₂ is not high enough to induce any detectable damage to cellular components [43••]. It is possible that compatible solutes protect the protein-synthesizing machinery against various kinds of stress thereby maintaining conditions under which repair processes occur more rapidly than damaging processes. This hypothesis should be examined further in future studies.

Conclusions and perspectives

The production of transgenic plants that can accumulate various compatible solutes, in particular plants of model species such as Arabidopsis and tobacco, has allowed this stress defense mechanism to be extended to crop plants, such as rice, potato, and sugar beet, albeit with varying degrees of success. In the next few years, it is likely that stress-tolerant plants that accumulate compatible solutes will be generated in additional species. Field tests of these transgenic crops under stress conditions will help to verify their potential utility in crop-improvement programs. The genes that have been tested in attempts to engineer the production of compatible solutes may not be equally suitable for efforts to improve stress tolerance. Among the seven types of compatible solute that have been produced in transformed plants, mannitol, sorbitol, and trehalose might be of only limited utility because of the detrimental effects associated with high concentrations of these compounds.

For practical application in crop plants, the capacity for synthesis of a particular compatible solute must be improved further. Increases in the levels of expression of transgenes, and in the availability of the appropriate substrate in a specific subcellular compartment, should facilitate generation of plants that produce elevated levels of specific compatible solutes and that, as a consequence, are more tolerant to various stresses.

Progress might also be made by combining different strategies that are individually effective in enhancing stress tolerance. Targets for this approach include genes whose products are involved in the biosynthesis of compatible solutes; stress-induced genes, such as the COLD-Regulated (COR) and Late Embryogenesis-Abundant (LEA); and genes for regulatory proteins, such as stress-inducible transcription factors [44]. The acclimation of plants to abiotic stress is a complicated response and probably involves many genes, with each individual gene playing a unique role in determining the overall tolerance of stress. The combination of various components might enhance all of the tolerance-related traits together in a single plant. Such enhancement would have a significant impact on attempts to increase the stress tolerance of agriculturally important crop plants.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
**of outstanding interest


Application of exogenous GB significantly increased chilling tolerance in suspension-cultured cells and seedlings of a maize genotype that does not accumulate GB even during salt stress. At 4°C, the amount of MDA, the end-product of lipid peroxidation, was higher than that in maize cells that had been grown in a warm environment. Lipid peroxidation during chilling, expressed in terms of the production of MDA, was significantly greater in untreated controls than in GB-treated cells. These results suggest that the GB-enhanced tolerance to chilling might be due, in part, to reduced lipid peroxidation of cell membranes in the presence of GB. It is also possible, however, that the reduction in lipid peroxidation might have resulted from reduced production of ROS via direct protection of organelles that are thought to produce ROS under stressful conditions (i.e. mitochondria and chloroplasts).


A review of recent transgenic approaches to the enhancement of stress tolerance in plants by metabolic engineering of GB biosynthesis. The authors summarize known pathways for the biosynthesis of GB, and discuss the introduction of these pathways into plants.


Resembling [7**], this review highlights recent advances in the engineering of GB synthesis in cyanobacteria and in various plants. The authors also discuss the possible role of GB in stress tolerance in vivo.


A review summarizing recent progress in the genetic manipulation of GB synthesis, with special emphasis on the relationship between protective effects in vivo and those documented in vitro.


The authors describe the metabolic steps for oxidation of choline to GB into two extreme halophiles. The authors identified a three-step series of methylations from glycine to GB, which are catalyzed by two methyltransferases that have partially overlapping substrate specificity.


The authors present a detailed model of the labeling kinetics of choline metabolites. They demonstrate that the import of choline into chloroplasts limits the synthesis of GB in the chloroplasts.


33. Native PCS is subject to feedback inhibition by proline. The authors used site-directed mutagenesis to replace Phe at position 129 by Ala in PCS of V. aconitifolia. The mutant enzyme (PCS F129A) was no longer subject to feedback inhibition. Plants expressing PCS F129A accumulated about twice as much proline as those expressing wildtype PCS. This difference was further accentuated in plants treated with NaCl. The elevated levels of proline significantly improved the salt tolerance of transgenic seedlings. Increased levels of proline also reduced the production of free radicals, as measured in terms of MDA production. These findings indicate that, in addition to serving as an osmolyte, proline might play a role in reducing the oxidative stress that is brought on by osmotic stress.


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The authors examine whether ROS act primarily by directly accelerating damage to photosystem II (PSII) or by inhibiting the repair of the photodamage to PSII in vivo. They provide evidence that increased intracellular concentrations of ROS inhibit repair of damaged PSII. In experiments involving the synthesis of labeled protein in vivo, and Northern and Western blotting analyses, they demonstrate that ROS inhibited the synthesis of the D1 protein primarily at the translational level, at both the polypeptide-initiation and polypeptide-elongation steps.


The accumulation of GB due to transformation with the codA gene dramatically improved the survival of mature plants at freezing temperatures. However, several COR genes (COR6.6, COR15a, COR47, and COR78), which have been implicated in the development of freezing tolerance, were apparently not responsible for this enhanced tolerance to freezing. There were no significant differences in the levels of COR expression between the transgenic and wildtype plants.


