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# 甜菜碱提高植物抗盐性的作用机理 及其遗传工程研究进展\*

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**摘 要** :系统地讨论了甜菜碱在提高植物抗盐性中的作用机理及其国内外研究进展, 并对甜菜碱生物合成过程中关键酶及其遗传工程的研究进展进行了综述。提出在进一步弄清甜菜碱提高植物抗盐性作用机理的基础上, 应在重要作物中开展甜菜碱合成相关基因的导入, 以期获得耐盐植物新品种。

**关键词** 甜菜碱 植物抗盐性 作用机理 遗传工程 研究进展

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## Mechanism for Glycinebetaine to Improve Plant Salt Resistance and Its Research Advances in Genetic Engineering

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**Abstract** :The paper systematically discusses the mechanism for glycinebetaine to improve plant salt resistance and its research advances in genetic engineering at home and abroad as well as summarizing the research progresses about the key enzymes and their genetic engineering in glycinebetaine biosynthesis. It suggests that on the basis of further understanding the mechanism for glycinebetaine to improve plant salt resistance, the transformation of the genes relating to glycinebetaine biosynthesis should be carried out in major crops so that new plant varieties resistant to salt can be obtained.

**Key words** glycinebetaine ;salt resistance mechanism ;genetic engineering ;research advances

Salt stress is a global problem which restricts the normal growth and development of plants, decreases crop production and even exterminates some plant species<sup>[1]</sup>. Under the condition of salinity, plants can adapt themselves to the environmental changes by self-regulation. At the same time, the damage caused by salt stress can be mitigated

by external factors in some extent<sup>[2]</sup>.

Many previous research works indicate that plant cell can synthesize and accumulate some small osmotic molecules under some stresses, such as drought and salt. These small molecules can decrease cellular osmotic pressure and enhance water absorption, which is generally defined as osmotic

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adjustment (OA), a very important mechanism in plants<sup>[3]</sup>. In the process of OA, osmolytes naturally synthesized and accumulated in plant cells are mainly two groups. One is inorganic ions, such as  $\text{Na}^+$  and  $\text{K}^+$ , absorbed from the external environment; the other group is compatible solutes synthesized naturally by plants themselves. The most common compatible solutes synthesized in plants include polyol (mannitol and inositol), sugar (sucrose and trehalose), amino acid (proline) and their derivative (glycinebetaine)<sup>[4]</sup>. Glycinebetaine, also named betaine, has been focused on by many researchers because it is an effective compatible osmolyte in plants subject to salt stress<sup>[5]</sup>. In many plants, especially in the Chenopodiaceae and Gramineae, plant cells accumulate a large amount of GB when subject to drought and salt stresses and there is a positive correlation between the GB accumulation level and stress resistance ability in plants<sup>[6]</sup>.

Glycinebetaine (N,N,N-trimethylglycine; betaine) is an amino acid derivative that is naturally synthesized and accumulated in bacteria, fungi, algae, animals and many plant families<sup>[5,7,8]</sup>. It is synthesized from choline oxidation to betaine aldehyde in the chloroplast stroma of higher plants by a soluble ferredoxin-dependent choline monooxygenase (CMO)<sup>[9]</sup>. Oxidation of betaine aldehyde to glycinebetaine is catalyzed by  $\text{NAD}^+$ -dependent betaine aldehyde dehydrogenase (BADH; EC 1.2.1.8)<sup>[8]</sup>. GB protects higher plants against salt/osmotic stress by many different ways which include: maintaining osmotic adjustment<sup>[10]</sup>, stabilizing many functional protein units<sup>[11]</sup>, and stabilizing membrane integrity<sup>[12]</sup> and quaternary structures of complex proteins<sup>[13]</sup>. Besides, GB can affect antioxidant enzyme activities<sup>[14]</sup>.

## 1 Physiological and biochemical effects of GB on enhancing plant salt resistance

### 1.1 GB assistance in regulating osmotic adjustment of the plant cell

As an osmolyte, GB itself can regulate the cell osmotic pressure of plant under osmotic stress. Water deficiency caused by salt stress is a common phenomenon in plants. Water deficiency often leads to metabolic disorders. Leaf relative water content (RWC) and osmotic potential ( $\psi_s$ ) are two parameters often used to estimate water status in plants. In their research, Guo *et al.*<sup>[4]</sup> reported that at the same concentration of NaCl, adding  $15 \text{ mmol} \cdot \text{L}^{-1}$  GB in the incubation medium could increase leaf RWC and decrease  $\psi_s$  respectively compared to the control. In addition, GB can also stimulate other osmolytes in plant cells. In the work of Zhang *et al.*<sup>[2]</sup>, they found that exogenous application of appropriate concentration of GB could increase the content of soluble sugars, proline and soluble proteins in wheat leaves and roots under NaCl stress. Xu *et al.*<sup>[15]</sup> applied exogenous GB to *B. rassaica chinensis* at  $15 \text{ mmol} \cdot \text{L}^{-1}$  concentration. It not only increased the proline content, leaf weight and chloroplast content remarkably, but also decreased the membrane permeability of the seedling under different NaCl concentrations.

### 1.2 Influences of GB on ion absorption and transportation in plant cells

On salt stressed condition, many ions can be accumulated in plant cells, which affects the plant normal physiological and biochemical activities in two aspects. One is the toxic effect of ions; the other is that the existence of special ions may cause plant malnutrition<sup>[16]</sup>. It is important for plants to keep low  $\text{Na}^+$  and low  $\text{Na}^+/\text{K}^+$  ratio to tolerate salinity<sup>[17]</sup>. Zhang *et al.*<sup>[2]</sup> reported that GB enhanced not only the root absorption selectivity but also the transportation selectivity of root to leaf. Xu *et al.*<sup>[15]</sup> reported that the exogenous spray of GB at the concentration of  $15 \text{ mmol} \cdot \text{L}^{-1}$  could decrease the  $\text{Na}^+$  accumulation and  $\text{Na}^+/\text{K}^+$  ratio in *B. rassaica chinensis* root and leaf significantly; in addition, exogenous spray of GB ( $0\sim 20 \text{ mmol} \cdot \text{L}^{-1}$ ) decreased approximately 300% the permeability of plasma membrane of *B. chinensis* leaf under salt stress when compared to the salt treated group alone. Furthermore, the exogenous spray of GB ( $15$

mmol · L<sup>-1</sup>) increased the absorption selectivity of Na<sup>+</sup> and K<sup>+</sup> of roots, i.e. GB accumulating in roots enhanced the ability of excluding Na<sup>+</sup>. However, GB had no significant effects on root transportation selectivity. The ion absorption is partly controlled by the permeability of plasma membrane. The decrease of permeability caused by GB treatment could avoid the abnormal metabolism of plant and maintain its normal growth and development by restraining the excessive Na<sup>+</sup> from influxing into plant cells. In addition, some membrane proteins play a significant role in selective distribution of ions within the plant cells. H<sup>+</sup>-ATPase is one of the most important proteins, which generates the H<sup>+</sup> electrochemical gradient that drives ion transportation<sup>[18]</sup>. Xu *et al.*<sup>[15]</sup> found that exogenous GB also increased the H<sup>+</sup>-ATPase activity under both stress and normal condition.

### 1.3 Effect of GB on maintaining the quaternary structure of complex proteins stable

Thylakoid membranes of chloroplasts contain the oxygen-evolving photosystem II (PS II) complex which is composed of a number of intrinsic proteins and extrinsic proteins with relative molecular weights of 18, 23 and 33 kD. In their work, Murata *et al.*<sup>[19]</sup> found that the incubation of PS II particles with 1.0 mol · L<sup>-1</sup> NaCl resulted in dissociation of almost all of the 18 and 23 kD proteins and so decreased the oxygen-evolving activity. But the presence of 1.0 mol · L<sup>-1</sup> GB in the incubation medium inhibited the dissociation of the intrinsic proteins from the complex and increased the oxygen-evolving ability. In addition, Stamatakis *et al.*<sup>[20]</sup> found that PS II particles, which were rendered inactive as a result of NaCl-induced depletion of the 9 kD protein, acquired the oxygen-evolving competence if resuspended in the presence of GB.

### 1.4 Effects of GB on the antioxidative system in plants under salt stress

A large number of evidences suggested that environmental stresses, especially drought and salt stress, increased the production of activated oxygen radicals (AOR) by enhancing leakage of electrons from electronic transport chains in the chloroplasts

and mitochondria to molecular oxygen<sup>[21]</sup>. It is already known that cytotoxic AOR can destroy normal metabolism through oxidative damage of lipids, proteins, and nucleic acids<sup>[22]</sup>. Membrane injury induced by salt stress is related to an enhanced production of highly toxic AOR, as well<sup>[23]</sup>. Therefore, AOR must be scavenged for maintenance of normal growth. Oxidative damage in the plant tissue was alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant mechanisms<sup>[24,25]</sup>. Among the enzymes involved in alleviating oxidative damage, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (AP) and glutathione reductase (GR) are usually used to estimate the salt tolerance ability of plants under stresses. Recent study of Tijen *et al.*<sup>[26]</sup> illustrated that application of exogenous GB (15 mmol · L<sup>-1</sup>) could decrease the activities of SOD, AP, CAT and GR in salt tolerant rice cultivar Pokkali and the activity of POX in salt sensitive cultivar IR-28 respectively, whereas it increased the activities of CAT and AP in IR-28 when compared to the salt treated group alone. In addition, the lipid peroxidation levels of both cultivars under salt stress showed a decrease with GB treatment. GB treatment protected rice from salinity-induced oxidative stress. Guo *et al.*<sup>[4]</sup> found that in the salt stressed condition, exogenous application GB (15 mmol · L<sup>-1</sup>) could increase the activities of SOD, APX and POD in wheat seedling. But the activity of CAT is not influenced by GB treatment.

## 2 Genetic engineering of GB synthesis in plants

A large number of evidences suggest that GB is a multi-functional molecule to enhance salt tolerance in plants. But many important farming crops, such as rice, tomato, potato and tobacco can not synthesize GB. These plants have long been a potential target for engineering the GB metabolic pathway and thus for stress resistance. As mentioned above, the synthesis of GB in higher plants includes two steps: the first step is choline oxida-

tion to betaine aldehyde catalyzed by a soluble ferredoxin dependent choline monoxygenase (CMO)<sup>[18]</sup>, the second step is the oxidation of betaine aldehyde to GB catalyzed by NAD<sup>+</sup>-dependent betaine aldehyde dehydrogenase (BADH; EC 1.2.1.8)<sup>[17]</sup>. Gene technological introduction of the GB synthesis pathway into non-halotolerant plants would result in the accumulation of GB and enhancement of their tolerance to salt stress

### 2.1 Genetic engineering of BADH gene

BADH catalyzes the oxidation of betaine aldehyde to GB. Transgenic rice plants expressed a barley gene for BADH conferred higher tolerance to salt, low temperature and higher temperature stresses than wild type plants<sup>[27]</sup>. Luo *et al.*<sup>[28]</sup> transferred tobacco with *BADH* of spinach, the activities of SOD, CAT, POD and GR were higher compared with control. Jia *et al.*<sup>[29]</sup> transformed the *BADH* gene cloned from *Atriplex hortensis* and controlled by 35S promoters of the cauliflower mosaic virus, into a salt sensitive tomato cultivar, Bailichun. Polymerase Chain Reaction (PCR) and Southern hybridization analysis demonstrated that the *BADH* gene had been integrated into the genome of tomato. Transgenic tomato plants showed significantly higher levels of mRNA and BADH enzyme activity than wild-type plants and the transgenic plants exhibited tolerance to salt stress. These plants could grow normally at salt concentration of 120 mmol · L<sup>-1</sup>.

### 2.2 Genetic engineering of betA gene

Bacterial choline dehydrogenase (cdh) catalyzes not only the oxidation of choline to betaine aldehyde, but also the second step to GB. It is thus the most useful enzyme introduced into new species<sup>[30]</sup>. Bhattacharya *et al.*<sup>[31]</sup> transferred bacterial *betA* gene encoding cdh to cabbage through *Agrobacterium* mediated transformation of hypocotyl explants. Transgenic status was established through Southern hybridization and mRNA expression in the shoots. The transformants exhibited higher tolerance to NaCl stress compared to untransformed parent plants.

### 2.3 Genetic engineering of codA gene

The *codA* gene is for choline oxidase. Choline oxidase (E.C. 1.1.3.17) catalyzes the oxidation of choline to betaine (N,N,N-trimethylglycine; betaine). Hayashi *et al.*<sup>[32]</sup> transformed *Arabidopsis thaliana* with the *codA*. It enabled the plant to accumulate GB and enhanced its tolerance to salt and cold stress. Prasad<sup>[33]</sup> transformed *Brassica juncea* with *codA* gene from *Agrobacterium globiformis*. The *codA* gene was targeted into the chloroplasts to enable the transformed plants to synthesize glycinebetaine (GB) in their chloroplasts. Accumulation of glycinebetaine in the chloroplasts led to increased tolerance of the PS II in transgenic lines to high light intensity under unstressed and stressed (salt as well as low temperature stress) conditions. Enhanced tolerance is due to increased protection as well as accelerated recovery of the PS II from a photo-inactivated state by glycinebetaine.

## 3 Conclusion and prospect

GB is naturally occurring compatible solute, which is widely distributed in plants, animals and bacteria. GB protects cells from stresses by maintaining an osmotic balance with the surrounding environment, stabilizing functional protein units, stabilizing membrane integrity and quaternary structures of complex proteins. At the same time, GB can influence the antioxidative system of plants under stresses. There is a linear relationship between the GB accumulation level and salinity tolerance in plants. Many previous research works about GB have been focused on its physiological functions of osmotic protection, while few attentions have been paid on its functions to increase antioxidative ability and anti-photoinhibition ability of plants under stresses. Plant antioxidative system is very sensitive when plants are subjected to stresses<sup>[34]</sup>. The changes of plant antioxidants are regarded as very important indicators for plant stress tolerance. Therefore, the physiological mechanism of GB on antioxidative system in plants under stresses should be emphasized on in the future research works. As we all know, photosynthesis is

one of the key metabolic pathway that is responsible for growth and development of plants<sup>[35]</sup>. Many stresses, such as salt and drought, often lead to the destruction of photosynthetic organs, and then the capability of plants to utilize the light absorbed by them declined significantly<sup>[36]</sup>. This reduction in photosynthesis efficiency could be due to or leads to photoinhibition. As far as we know, little has been reported about GB and photoinhibition. So, we suggest that future research works should be emphasized on it. In the field of GB synthesis genetic engineering, many works have been done in recent years and a lot of useful genes have been cloned and transferred to plants, such as *BADH*, *betA* and *codA* etc. The transformation of these genes to plants increased the salinity tolerance of plants and decreased the damage caused by salt stress. However, there still exists many problems. Many gene transformation research works are only confined to laboratory level, little has been applied to field production. Most of the gene transformation works

have been carried out only on model plants. Furthermore, enhancement of plant salt tolerance by single gene transformation is efficient for some genes or plants, but not for all. In addition, the expression of exogenous genes is usually unsteady. To solve the problems above, future studies should be concentrated on some major crops, such as wheat, rice, maize etc. And better agronomic character cultivars should be chosen as the receptors. Transgenic plants should be applied to field production as quick as possible. In some plants, multi-genes should be transformed to enhance their salt tolerance, such as the combination of GB synthesis gene and regulative gene. In addition, we should explore deeper mechanisms of GB on plant salt tolerance, clarify its complex physiological and biochemical processes and get to know its molecular basis. With the further understanding of them, we will obtain salt tolerant plant cultivars by the combination methods of gene engineering and conventional breeding in the near future.

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