

PLANT CELLULAR OSMOTICA

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ABSTRACT

To cope with the water deficit resulting from saline environment, plant cells accumulate three kinds of osmotica: salts, small organic solutes and hydrophillic, glycine-rich proteins. Salts such as NaCl are cheap and available but has ion toxicity in high concentrations. Small organic solutes are assistant osmotica, their main function is to protect cytoplasmic enzymes from ionic toxicity and maintain the integrity of cellular membranes. Hydrophillic, glycine-rich proteins are the most effective osmotica, they have some characteristics to avoid crystallization even in high concentration, but because they are expensive they are not as commonly used as salts or organic solutes. In addition there is the question of whether the genetic information for growth in saline environment is present in all kinds of plants, both halophytes and nonhalophytes.

1. INTRODUCTION

To survive in highly saline condition, the plant cells must accumulate some osmotica to counter the water deficit. Which osmoticum is more effective in maintaining cellular water in plants exposed to salinity, or in other words, why the plant cells accumulate it or them instead of others, is an inescapable question. To answer this, it is necessary to discuss if the genetic information for growth in saline condition is present in all plant species, especially in salt-sensitive species. If it is true, the mechanism of salt tolerance must be complex and the choice of any osmoticum for salt-sensitive plant responding to salt stress must be concerned both the characteristics of the osmoticum and the physiologies of the plants. If it is not the fact, the accumulation of any osmoticum is a separate feature and the choice of it is a stochastic event.

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2. CONGENITAL OR EPIGENETIC

From the reviews (Flowers *et al.*, 1977; Gorham *et al.*, 1985; Greenway *et al.*, 1980; Hasegawa *et al.*, 1986; Lerner, 1985; Yeo, 1983) it can be seen that the genetical salt adaptation or tolerance of plants should not be attributed to simple increments of separate mutations because of the ingenious internal relationship like ion accumulation and compartmentation, and the compatible solutes and corresponding enzymes structure (Manetas *et al.*, 1986). Also from those reviews, we know that the salt adaptation of the plants involve a lot of physiological and anatomical changes meanwhile there are many differences between halophytes and nonhalophytes. Thus if the salt-sensitive plants do not have the genetic information for salt-resistance, making them grow in saline condition through mutations is like to produce a frog from a fish with the help of mutagens in a laboratory. The result of Binzel *et al.* (1985) established that tobacco cells arising from a salt-sensitive plant can survive and grow under conditions of extreme salinity, and the adaptation involved considerable osmotic adjustment with increased turgor but reduced cell expansion. Lerner (1985) discussed this in his review. In his opinion, the transformation of salt sensitive cells into salt-resistant cells is most probably due to a phenomenon similar to differentiation, because it occurs much too rapidly to be the result of a mutation.

This can be considered in the light of recapitulation theory. All the vertebrate embryos develop pharyngeal pouches, which give rise to gill in fish and amphibians, but during further development either disappear or are modified to form different structures in birds, reptiles and mammals. It is believed that embryogenesis recapitulates evolution history. Even though human beings do not have gills in the adult stage, they have at least part of the ancestral genetic information to build gills. This information has not been lost. If recapitulation theory hold the truth, one can image that a lot of other genetic information of our early ancestors may be also retained though perhaps no one knows the features that represent it during human development. According to the well accepted evolution theory, the terrestrial plants are descendants of the ancient halobios, which adapted to the highly saline condition --- sea water. Compared with the animals discussed above, the terrestrial plants might have the genetic information for growth in saline condition and express it at some developmental stage like late embryogenesis. At present, biologists could not use the recapitulation theory accurately. It is not clear that whether all or some of ancestral characters can recapitulate, and if not all, which of them. The genetic information of the recapitulated characters had been surely modified at least in regulating sequences of gene expression. Halophytes and nonhalophytes respond to environmental salt stress in similar ways e.g. accumulating certain amount of salt and/or small organic solutes, but in most cases the nonhalophytes fail to grow in saline condition. Overexpression of genes that synthesize small organic solutes has been demonstrated to increase salt tolerance for some degree in transgenic plants (Kishor *et al.*, 1995). The fact that tobacco cannot grow well in saline condition is not because it cannot produce proline, but because it cannot produce enough proline. It may be concluded that modern salt-sensitive plants have most of their ancestral genetic information for salt tolerance, but regulating elements may be modified. As the salt tolerance involves a complex mechanism, great hope should not be placed on such biotechniques that introduce one or two structural genes into salt-sensitive crop plants.

On the premise that the genetic information for salt tolerance is inherent in all plant species, every detail of the physiological tolerance must be coordinated.

3. SALTS AND SMALL ORGANIC SOLUTES

Before discussion on plant cellular osmotica, the predictions about plant processes made by Bloom and coworkers (Bloom *et al.*, 1985) should be mentioned first. Plants adjust physiologically to changes in resource availability to reduce extreme exchange ratios. The resource was defined as materials in the environment or within the plant required for a process, and the exchange ratio is the relative cost of two resources. For plants under salt stress, salt, small organic solutes and some proteins are all resources required for osmotic adjustment. Yeo (1983) attempted to give an accurate calculation for the cost of osmotica in the term of ATP. ATP is often considered as the sole currency in biological system, however it is obvious that more efforts must be used to gain one unit of ATP for plants growing in shaded condition than those in sunny condition. Biologists have not found a perfect currency in biological systems as money in economic field (Bloom *et al.*, 1985). Apparently the costs of osmotica vary with the abundance of different resource e.g. in N-deficient condition, plants will preferentially accumulate sugars instead of amino acids as osmotica if there is not great difference in their ability to bind water. Attention should be paid on the fact that salt tolerance mechanisms had been evolved in ancestral plants living in the ancient sea, the heterogeneous palaeoecological habitats might lead to several different strategies for plants to survive in saline condition, meanwhile temporal heterogeneity might force ancestral plants to utilize two or more strategies to various extents. It is difficult to accurately estimate the relative costs of different osmotica in ancient sea, but it is sure that salts had not only great but also permanent availability. It was permanent availability that made it possible for plants to genetically evolve mechanisms to avoid the harmful effects of osmotica, like compartmentation of ions into vacuoles and the harmonization between proline and enzyme structure (Manetas *et al.*, 1986), which are also indispensable parts of the salt tolerance mechanism. Plant cells which accumulate salts as main osmotica, must compartment a certain proportion of salts into vacuoles because of the ion toxicity of high concentration of salt. The total cost of salts is the energy used in transporting salts across both plasma membrane and vacuolar membrane. For the small organic solutes like proline large quantities of energy had been used in their synthesis, and if they are used in root cells, energy needed for transportation is also absolutely necessary. Most plants selected salts as primary osmotica probably because of the above characteristics: great, permanent availability and not too high costs.

In general, enzymes and metabolic processes such as protein synthesis have a relatively narrow range of ion concentrations and compositions, at which they show optimal activity. Enzymes from species differing in salt tolerance did not show apparent difference in response to salt (Greenway *et al.*, 1972). With the increasing salinity in the environment, more osmotica are required for plant cells to survive. Apparently more salt in plant cells, the more serious the ion toxicity is. Alternate compatible solutes are more suitable for this case. The accumulations of various small organic solutes within the salt stressed plant cells have been widely reported (Cusido *et al.*, 1987; Gorham *et al.*, 1981; Greenway *et al.*, 1980; Hasson *et al.*, 1983; Sudhakar *et al.*, 1993; Yancey *et al.*, 1982). Although salts are principal components of osmotic adjustment, small organic solutes also make significant contributions (Binzel *et al.*, 1987; Lerner, 1985).

However, several other kinds of environment stresses such as drought (Sing *et al.*, 1972), metals (Bassi *et al.*, 1993), cold and freeze (Naidu *et al.*, 1991; Parker *et al.*, 1962; Li *et al.*, 1966) also result in the accumulation of proline and other small organic solutes

in many plant species. At least water-deficit does not happen critically under metal stress, so osmotica do not seem necessary. This suggests small organic solutes have other functions than osmotica. Numerous studies in the past have shown that small organic solutes are not only non-toxic, but also protect the enzymes at least of the same species (Manetas *et al.*, 1986) in vitro against NaCl, heat, dilution, hydroxyl, cold and freeze (Carpenter *et al.*, 1988; Manetas *et al.*, 1986; Paleg *et al.*, 1981; Shirahashi *et al.*, 1978; Smirnoff *et al.*, 1989; Stamatakis *et al.*, 1988). In addition, small organic solutes had been demonstrated to stabilize biological membrane (Jolivet *et al.*, 1982; Rudolph *et al.*, 1986). From these data, we may conclude that small organic solutes not only act in osmotic adjustment to some extent, but also protect the enzymes and other macromolecules and maintain the membrane integrity against the biological unfavourable consequences of stress-induced thermodynamic perturbation. Some evidences indirectly support this proposition. The proline content of sorghum leaves was linearly related to the total monovalent cation content above threshold concentration (Weinberg *et al.*, 1982). In salt stressed potato cell suspension cultures, more than 80% of the proline are not in vacuoles but restricted to the cytoplasm (Fricke *et al.*, 1990) where ionic toxicity is critical.

Severe osmotic stress also happens in freezing cells because of disappearing of intracellular and extracellular water into ice crystals. Freezing stress differs from salt stress in its temperature stress although they share the similarity of osmotic stress. So freezing tolerance mechanism must have evolved separately in evolution. The non-synchronization between the great availability of salts and freezing stress might be responsible for the accumulation of small organic solutes to bind water instead of salts.

Small organic solutes as compatible solutes can counter the water deficit resulting from salinity- or water-stress. But if only one of them acts as osmotica in dormant seeds or in the plant cells exposed to an extremely water-deficit condition, they need to accumulate to an extremely high concentration. In consequence they tend to crystallize themselves, which in turn leads to the limit of their effectiveness in osmotic adjustment. Thus plants able to accumulate two or more osmotica simultaneously would be better able to cope with severe desiccation than plants able to accumulate only one of them. Intriguingly, severe desiccation leads to accumulation of uncharge solutes like trehalose or sucrose, instead of charge ones like proline or betaines in mature plant seed, fungal spores and dormant embryos of *Artemia salina*, which, according to Bakar *et al.*, is to maintain dielectric constraints (Bakar *et al.*, 1988; Clegg, 1965 and references therein).

4. HYDROPHILIC GLYCINE-RICH PROTEINS

In the final stage of maturation, seeds lose most of their water. Many macromolecules would irreversibly lose most of their biological activity on removal of water without the action of certain cellular components that prevent complete dehydration of macromolecular structures. In this critical stage, some similar proteins and their mRNAs accumulate in embryo tissues of seeds, and remain constant in resting seeds, normally disappear at the onset of germination. Most of these proteins such as Em, Rab, LeaD11 and LeaD13 are extremely hydrophilic and glycine-rich. The concept of resistance to extreme osmotic stress was first proposed by Lane and coworkers (Hoffman *et al.*, 1984; McCubbin *et al.*, 1985) as a function for the abundant Em polypeptides of wheat embryos and has subsequently been extent to encompass over other abundant proteins which accumulate in developing

plant embryos (Close *et al.*, 1989; Gómez *et al.*, 1988). Many experiments have demonstrated that these proteins are ubiquitous in higher plants and can be induced to high levels of expression in other tissues or at other stages of ontogeny than late embryogenesis and dormant seeds, by exogenous stimulation like abscisic acid, desiccation or salt stress (Bostock *et al.*, 1992; Close *et al.*, 1989, Galau *et al.*, 1986; Godoy *et al.*, 1990; Gómez *et al.*, 1988; Gulick *et al.*, 1992; Marcotte *et al.*, 1989; Morris *et al.*, 1990; Mundy *et al.*, 1988; Vilardell *et al.*, 1990). These genes are also expressed in salt tolerant plants.

Mesembryanthemum crystallinum, facultative halophyte, express certain Rab genes in response to saline treatments before it switches to crassulacean acid metabolism to reduce water loss (Skiver *et al.*, 1990). Now it may be concluded that the function of the hydrophilic, glycine-rich proteins are at least related to the tolerance of plant to salinity or desiccation. In the last part of this paper we will discuss whether these proteins can act as endogenous osmotica in osmotic adjustment.

The structure and function of these hydrophilic, glycine-rich proteins are a perfect coincidence. First, to bind water, these proteins should be soluble and have a high hydration potential. They are extremely hydrophilic and amino acid composition and sequence analysis revealed that they have a high proportion of polar and charged amino acid residues, while the proportion of nonpolar amino acid residues are very low, even dehydrins, Em, LeaD11, LeaD19 and TAS14 are tryptophan-free (Baker *et al.*, 1988; Close *et al.*, 1989; Godoy *et al.*, 1990; Gómez *et al.*, 1988; Litts *et al.*, 1987; Vilardell *et al.*, 1990) perhaps because the tryptophan side chain is very hydrophobic. McCubbin *et al.* (1985) discussed the hydration of Em proteins in detail, the result could extent to other hydrophilic, glycine-rich proteins. Second, to provide a matrix of bound water, they should be produced abundantly in the cytosol or vacuoles and not inclosed in other organelles or nuclei during the response to desiccation or salinity-stress. Neither Em proteins (Litts *et al.*, 1987) nor Lea proteins (Baker *et al.*, 1988) have functional leader sequences, which indicated that they are located in the cytoplasm. Immunoblots probed with antibodies raised against beta-galactosidase/rab21 fusion protein revealed that the Rab21 proteins were present only in the soluble fraction and not in crude organellar or nuclear fractions (Mundy *et al.*, 1988). Third, they should have a bigger surface than normal value, myoglobin cannot act as this character because most of its amino acid residues are covered and only a few residues have the opportunity to interact with the surrounding water. The best conformation for them is a random coil like β -casein. In randomly coiled conformations, not only polar and charged side chains but also part of the peptide backbone can bind water, while the latter has more powerful water affinity because of intramolecular interaction (Wolfenden, 1983). Physical properties of Em proteins had been characterized by McCubbin *et al.* (1985). The Em proteins have a much lower partial specific volume than typical, indicating that their peptide backbones are loosely folded, and they possess a high hydration potential. Its 70% random-coiled conformation had also been shown by circular dichroism. These proteins are glycine-rich. The fact that glycy residues have free rotation around the peptide bond suggests that these proteins have no thermodynamically preferred structure in aqueous solution, but rather may exist as amorphous random coils (Baker *et al.*, 1988). Last, acting as endogenous osmotica to counter water-loss, they should accumulate to a high concentration in the cytoplasm, and should have structural features to avoid crystallization and then their effectiveness would have no limitation. These proteins are glycine-rich so as discussed above they might exist as random coils. In addition they have a low proportion of cysteines. Even dehydrin (Close *et al.*, 1989), TAS14 (Godoy *et al.*, 1990), leaD113

(Baker *et al.*, 1988), pMAII19 (Gómcz *et al.*, 1988) Em (McCubbin *et al.*, 1985) proteins do not have any cysteines. This fact may be explained by the following. Disulphide bonds, which would stabilize proteins to a certain conformation, are very few while glycine content is very high in these proteins so they have no preferred structure in aqueous solution, and microheterogeneity may be caused by different conformations. Charged amino acid residues especially arginine have much higher water affinities than polar amino acid residues (Wolfenden, 1983), but they are not more abundant than polar amino acid residues in these proteins. The side chains of serine and threonine residues may be modified by phosphorylation or glycosylation, which in turn increase the hydration potential of the proteins. The proteins that have various phosphorylated or glycosylated forms that differ in their phosphorylation or glycosylation levels have microheterogeneity. Phosphorylation had been shown for the salt-induced TAS14 protein of tomato (Torres-Schumann *et al.*, 1991) as well as the Rab17 maize protein (Vilardell *et al.*, 1990). TAS14 protein has been reported to be present in different forms arising from post-translational phosphorylation (Torres-Schumann *et al.*, 1991). TAS14, Rab17, and other hydrophilic, glycine-rich proteins of rice, cotton, and barley include in their sequences the consensus sequence SED for phosphorylation by casein kinase II (Torres-Schumann *et al.*, 1991 and references therein) suggesting that phosphorylation might occur universally for all the hydrophilic, glycine-rich proteins to increase their water affinities and provide microheterogeneity.

We have presented a model which is most appropriate to bind water. However, other harmful effects may also happen resulting from severe desiccation like the mounting ion strength proposed by Baker *et al.* (1988). Subsets of the late embryogenesis abundant proteins may function in binding water or protecting cellular structures, or both of them to various extents, or even some other aspects. Thus, not all Lea proteins meet the above description, in addition description of the proportion of glycine, cysteine or tryptophan is qualitative instead of quantitative, for the proportion varies slightly among different proteins. Group 3 of Lea proteins containing alpha-helices (Dure *et al.*, 1989) have been demonstrated to increase tolerance of plants to water deficit and salinity if being constitutively expressed (Xu *et al.*, 1996). The helices of Lea proteins have been supposed to present a globular surface for the binding of ions (Baker *et al.*, 1988).

5. CONCLUSION

In conclusion, plant cells growing under saline condition, accumulate a controlled amount of salts as osmotica because it is available and economical. But excessive ions would increase the cellular ionic strength and then ionic toxicity would occur. Small organic solutes are compatible osmotica and have protective functions against ionic toxicity. Different osmotica (organic and inorganic) work in coordination avoiding crystallization caused by excessive high concentration of a single osmoticum. A group of proteins characterized by extreme hydrophilicity and glycine-richness are most suitable for osmotica in structures, but as regard to the energy used --- the synthesis of amino acids and the two high energy bonds broken for the synthesis of every peptide bond, they are two expensive. So the use of them in osmotic adjustment is not as common as salts or small organic solutes, particularly where the salt stress is not extremely critical. Coordination of different osmotica would result in the optimum integration of economization and effectiveness. The adaptation to salinity of any plants (including nonhalophytes) should not be the result of

simple increment of separate mutations in experiments, but the activation of the depressed genetic information, which had evolved in the long-term evolution of old age.

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