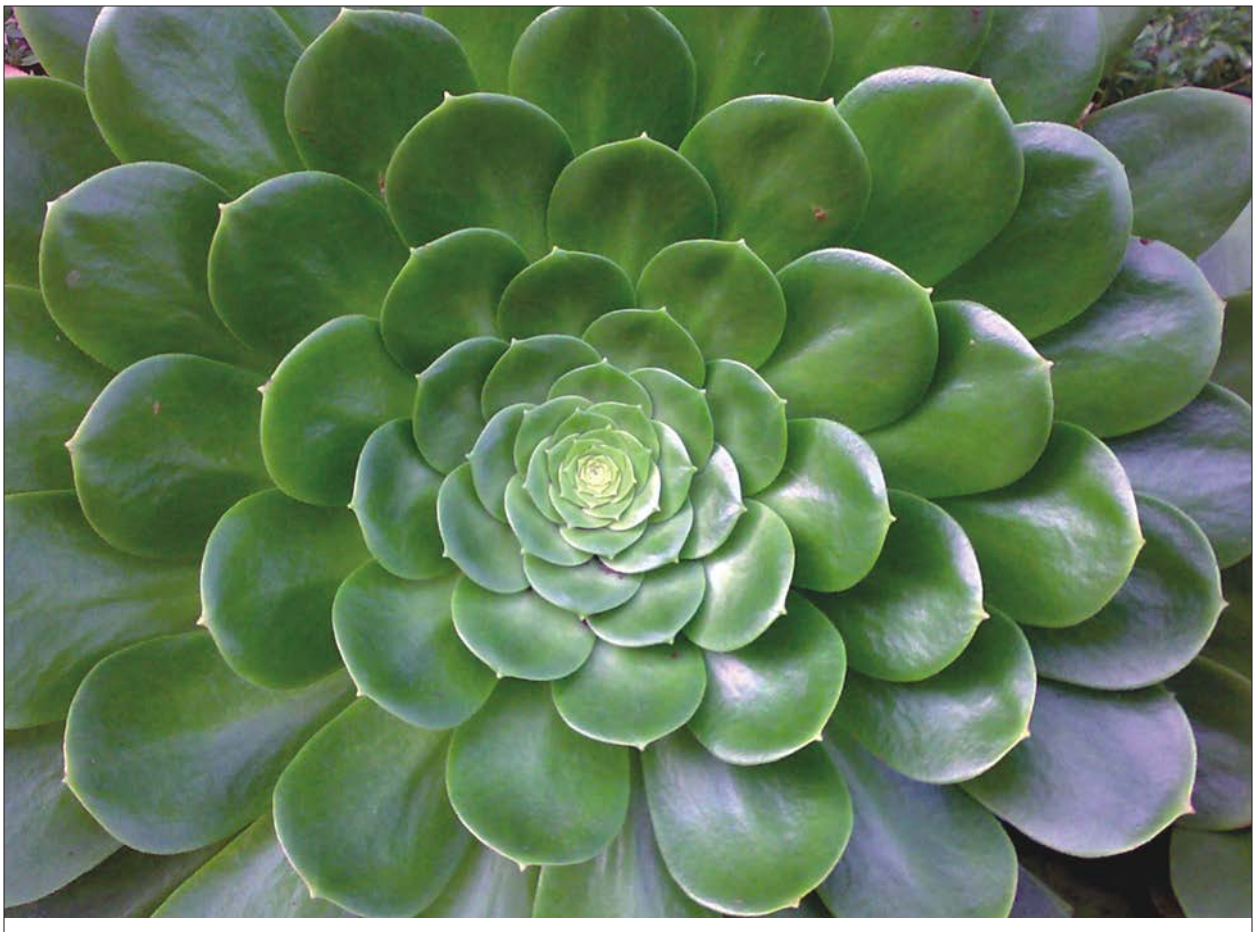

Journal of
Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 5 (4): 376-390, 2010
ISSN 1816-4951

Salinity Induced Programmed Cell Death in Plants: Challenges and Opportunities for Salt-tolerant Plants

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Abstract: Salt stress is one of the major abiotic stresses faced by plants, which adversely affect their productivity, affects large terrestrial areas of the world; the need to produce salt-tolerant crops is evident. Programmed Cell Death (PCD) plays an important role in mediating plant adaptive responses to the adverse environment such as salinity. Salinity that causes PCD in plant cells and is a substantial constraint to crop production. Two main approaches are being used to improve salt tolerance: (1) the exploitation of natural genetic variations, either through direct selection in stressful environments or through mapping quantitative trait loci and subsequent marker-assisted selection and (2) the generation of transgenic plants to introduce novel genes or to alter expression levels of the existing genes to affect the degree of salt stress tolerance. Cells subjected to salt stress showed a protective response which enabled them to survive. In the last few years, considerable progress has been made in the analysis of the transcriptome to study salt stress either alone or in combination with other abiotic stresses. However, there is no review that highlights the studies conducted to-date on salinity induced PCD in plants: Challenges and opportunities for salt tolerant plants. We believe that the present summary and perspective on salinity induced PCD in plants will provide a backbone to enable further studies on PCD occurs by salt stress and help to develop salt-tolerant plants through biotechnological strategies.

Key words: Salinity, programmed cell death, plants, salt tolerance, transgenic plants

INTRODUCTION

Agricultural productivity is severely affected by soil salinity because salt levels that are harmful to plant growth affect large terrestrial areas of the world. The damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations. It is estimated that 20% of the irrigated land in the world is presently affected by salinity (Yeo, 1999). This is exclusive of the regions classified as arid and desert lands (which comprise 25% of the total land of our planet). The loss of farmable land due to salinization is directly in conflict with the needs of the world population, which is projected to increase by 1.5 billion over the next 20 years and the challenge of maintaining the world food supplies. Although, famine in the world nowadays is caused by complex problems and not just by

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insufficient food production, the gains in agricultural output provided by the Green Revolution have reached their ceiling whereas the world population continues to rise. Therefore, increasing the yield of crop plants in normal soils and in less productive lands, including salinized lands, is an absolute requirement for feeding the world (Yamaguchi and Blumwald, 2005).

Salinity is a major environmental stress that causes PCD in plant cells and is a substantial constraint to crop production. Programmed Cell Death (PCD) is an important physiological process in both plants and animals and is responsible for the removal of redundant, senescencing and damaged cells (Palavan-Unsal *et al*., 2005; Hoeberichts and Woltering, 2003). In plants, programmed cell death is essential machinery for growth, development and plays a role in the response to stresses (Danon *et al*., 2000; Jones, 2000; Lam *et al*., 2001). Concurrently, PCD also acts as one manner of adaptations adopted by plants to survive biotic (Lam *et al*., 2001; Greenberg and Yao, 2004) and abiotic stresses such as salinity, cold stress, water logging and hypoxia (Drew *et al*., 2000; Kratsch and Wise, 2000; Huh *et al*., 2002).

The importance of studying and understanding programmed cell death in plants may assist in our ability to manipulate this process and thereby provide a useful tool in agricultural and post-harvest industries. For example, it may become feasible to increase the resistance of crops to various abiotic stresses such as drought and salinity. With global food production having to meet the demands of a growing world population, improving salt tolerance of crops is an important global priority and has become the focus for ongoing breeding efforts. This study is to review the programmed cell death of plants in response to salt stress and also the challenges and opportunities provided by recently developed functional tools for the development of salt-tolerant crops.

PROGRAMMED CELL DEATH IN RESPONSE TO ABIOTIC STRESSES

Plant cells and tissues exposed to variety of abiotic stresses that ultimately may result in their death. Abiotic stresses include toxins such as salinity, metals, herbicides and gaseous pollutants, including Reactive Oxygen Species (ROS), as well as water deficit and water logging, high and low temperature and extreme illumination. Plants show adaptations to the stress including mechanisms to tolerate the adverse conditions, to exclude the toxins or to avoid conditions where the stress is extreme. Abiotic stress may also result in stunted growth, followed by death of part or all of the plant (Palavan-Unsal *et al*., 2005).

PCD plays an important role in plant response to diverse abiotic stresses such as heat shock (Tian *et al*., 2000; Zhang *et al*., 2004), exposure to toxic chemicals (Sun *et al*., 1999), ozone (Overmyer *et al*., 2000; Rao *et al*., 2000; Pasqualini *et al*., 2003; Overmyer *et al*., 2005), UV radiation (Danon and Gallois, 1998) and hypoxia (Xu *et al*., 2004). Biochemical changes involving production of reactive oxygen species (Moeder *et al*., 2002), Ca release (Zuppini *et al*., 2003), proteolysis (DeJong *et al*., 2000) and ethylene biosynthesis (Spencer *et al*., 2003; Woltering *et al*., 2003) are found to actively participate in the plant PCD signal transduction.

Cell death in abiotic stress may therefore be part of a regulated process to ensure survival. Alternatively, it may be due to the uncontrolled death of cells or tissues killed by unfavorable conditions. PCD may be a part of an adaptive mechanism to survive the stress. Adaptation of plants to environmental conditions such as high light intensity or low humidity often involves covering their surfaces with layer of dead unicellular hairs. These cells are thought to go through PCD resulting in the formation of a protective layer that functions to block high irradiance and trap humidity (Greenberg, 1996). Table 1 summarizes the ultrastructural characterization of PCD in different abiotic stress conditions.

Table 1: Ultrastructural changes caused by various abiotic stresses (Evans, 2004)

Abiotic stress	Ultrastructural changes
Hypoxia-lysigenous	Chromatin condensation and DNA fragmentation
Aerenchyma formation	Organelle surrounded by membranes,
	Plasma membrane invagination and tonoplast degradation
	Cell wall degradation
Light radiation	Oligonucleosomal fragmentation of DNA
	Migration of nuclear contents to cell periphery
Mechanical stress	TUNEL positive material around nuclear periphery
	Oligonucleosomal fragmentation of DNA in chloroplast and nuclei
Cold stress	Chloroplast swelling, thylakoids distort and swell, grana unstuck and chloroplast lyse, nuclei swell, chromatin fragments, ER and golgi cisternae swell, cytoplasmic condensation occurs

Programmed Cell Death by Salt Stress

Programmed cell death (PCD) plays an important role in mediating plant adaptive responses to the adverse environment such as salinity. Salinity that causes PCD in plant cells and is a substantial constraint to crop production (Katsuhara and Kawasaki, 1996). Affenzeller *et al.* (2009) reported the salinity induced PCD in a freshwater green algae *Micrasterias denticulata*. They have shown that prolonged salt stress (24 h) led to degradation of organelles by autophagy, a special form of PCD where organelles are degenerated and enclosed by membranous structures derived from ER. This finding extends the phenomenon of salinity-induced PCD, previously reported in higher plants (Katsuhara, 1997; Katsuhara and Shibasaka, 2000; Lin *et al.*, 2005; Li *et al.*, 2007a, b) and yeasts (Huh *et al.*, 2002), to algal species. Importantly, DNA laddering, one of the hallmarks of PCD, was visible as soon as 1 h after the onset of NaCl stress (Affenzeller *et al.*, 2009) while previous reports suggested that at least 4 h of salinity treatment was needed (Li *et al.*, 2007a).

Affenzeller *et al.* (2009) also found that the observed DNA laddering occurred in NaCl but not in sorbitol-stressed cells. This indicates that the ionic rather then the osmotic component of salt stress led to the activation of the endonuclease resulting in PCD. To the best of my knowledge, the only previous report on such ionic specificity of PCD was by Huh *et al.* (2002). Although, the exact mechanisms beyond this specificity remain elusive, several lines of evidence suggest that changes in the cytosolic K^+/Na^+ ratio may be crucial for triggering PCD in living cells.

ROLE OF MITOCHONDRIA IN PCD BY SALT STRESS

It has long been known that mitochondria play a central role in the initiation of apoptosis in animal cells (Wang, 2001; VanLoo *et al.*, 2002). Recently, increasing evidences indicated that mitochondria also play a pivotal role in PCD in plants (Lam *et al.*, 2001; Jones, 2000). Firstly, in plant cells, especially in non-photosynthetic cells, mitochondria are the major source of Reactive Oxygen Species (ROS). Yao *et al.* (2004) found that ROS burst in mitochondria and outer membrane potential losses are early markers in *Arabidopsis thaliana*. Mitochondrial oxidative burst was also observed in other PCD systems (Vacca *et al.*, 2004; Tiwari *et al.*, 2002). High level of ROS causes damage to membrane protein thiols, leading to opening of the Permeability Transition Pore (PTP) and translocation of cytochrome c to the cytosol. Lin *et al.* (2006) investigated the involvement of mitochondrial Permeability Transition Pore (PTP) and cellular oxidation-reduction state in salt stress-induced Programmed Cell Death (PCD) of tobacco protoplasts and observed that the increased ROS and decreased mitochondrial membrane potential before the appearance of PCD.

Secondly, the energetic status of mitochondria is important for the initialization of PCD. After the mitochondria have been stimulated, the level of ATP may drive cells towards PCD (high ATP) or necrosis (low ATP) (Lemasters, 1999). Casolo *et al.* (2005) found that in soybean cell cultures, low concentration of H_2O_2 induces PCD, which is accompanied by a slight decrease in ATP and glucose-6-P levels, while at higher H_2O_2 concentration, cells become necrotic and the level of both decrease. In addition, ATP depletion after PCD induction in *A. thaliana* (Tiwari *et al.*, 2002) and tobacco BY-2 cells (Mlejnek *et al.*, 2003) has also been reported. Recently, Valenti *et al.* (2007) found that mitochondrial adenine nucleotide translocator, adenylate kinase and nucleoside diphosphate kinase are impaired in the early phase of PCD in tobacco BY-2 cells, which may contributed to ATP depletion. However, the molecular basis of ATP changes in mitochondria during the process of PCD is still largely unknown.

PROTEOMIC ANALYSIS OF MITOCHONDRIAL PROTEINS

In order to find more molecular elements related to PCD in plants, proteomic analysis was carried out. For example, Swidzinski *et al.* (2004) identified a number of proteins that are increased in relative abundance during heat-induced or senescence-induced PCD in Arabidopsis cell suspension culture, including several mitochondrial proteins such as aconitate hydratase, lipoamide dehydrogenase and voltage-dependent anion selective channel protein (VDAC). Mitochondria contain several pro-apoptotic molecules that activate cytosolic proteins to execute apoptosis, block anti-apoptotic proteins in the cytosol and directly cleave nuclear DNA. Mitochondria trap these pro-apoptotic proteins and physically separate pro-apoptotic proteins from their cytoplasmic targets (Gulbins *et al.*, 2003). Tsunezuka *et al.* (2005) analyzed PCD by proteomics using the rice lesion mimic mutant cdr2 and most of differentially expressed proteins were classified as metabolic enzymes, suggesting that PCD is associated with active metabolic changes. However, these studies focused on the proteome at whole cell level. It has been shown that the mitochondrial protein represents only a minor part of total proteins (Kruft *et al.*, 2001; Mihr *et al.*, 2001). Therefore a proteomic approach using total protein for the analysis of PCD has severe limitations due to large quantitative differences between abundant cellular proteins and rare mitochondrial polypeptides.

MECHANISMS FOR ION SPECIFIC SIGNALING DURING SALT STRESSING PCD

Abiotic pressures like salt stress and chemical insultance can impose limitations on crop productivity and also limit land available for farming, often in regions that can ill afford such constraints, thus highlighting a greater need for understanding how plants respond to adverse conditions with the hope of improving tolerance of plants to environmental stress (Joseph *et al.*, 2010). Under saline conditions, strong membrane depolarization caused by Na^+ uptake favours K^+ efflux via depolarization- activated outward-rectifying K čhannels (Shabala *et al.*, 2006). By contrast, isotonic mannitol or sorbitol solution causes significant membrane hyperpolarization, resulting in increased K+ uptake (Shabala *et al.*, 2000; Shabala and Lew, 2002). This will result in a dramatic difference in cytosolic K^+ level between these types of stresses (Shabala and Cuin, 2008).

Several plant hormones (e.g., ET, JA, SA, ABA, GA) may exert their, respective effects on plant PCD through the regulation of ROS accumulation. Recently, heterologous expression of the animal anti apoptotic CED-9 gene was shown to increase plant salt and

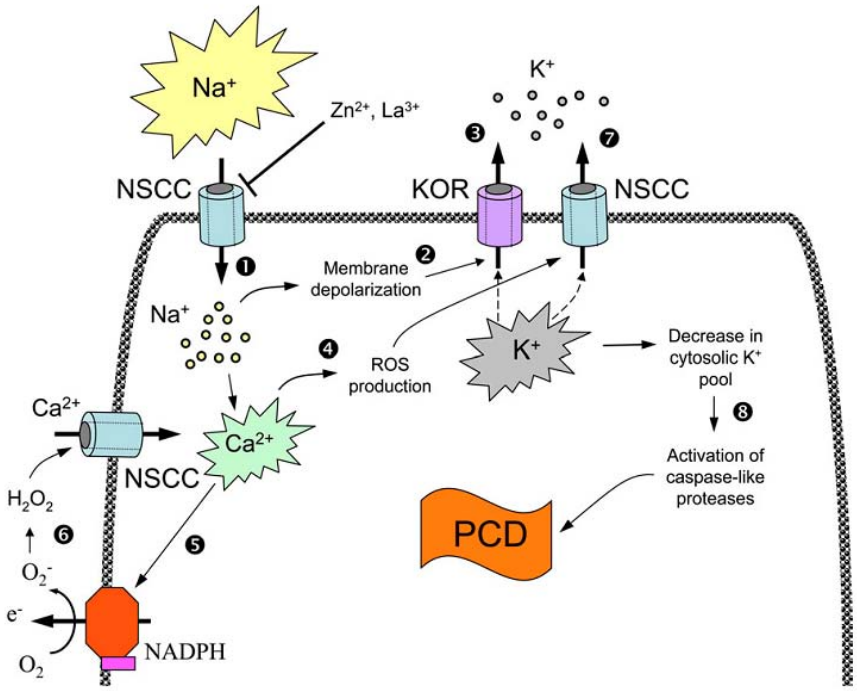

Fig. 1: The proposed model of ion specific signaling during PCD in plants. KOR, depolarization-activated outward-rectifying K+ channel; NSCC, non-selective cation channel; PCD, programmed cell death; ROS, reactive oxygen species (Shabala, 2009)

oxidative stress tolerance by altering K^+ and H^+ flux patterns across the plasma membrane of tobacco mesophyll cells (Shabala *et al*., 2007), pointing to ROS-regulated ion channels as an important component of the PCD machinery in plants. The following model was suggested by Shabala (2009) to explain the ion specific signaling during PCD in plants (Fig. 1).

- Under saline conditions, Na^+ enters the cell through NSCC
- Causing a significant membrane depolarization
- Resulting in a massive K^+ leak from the cell through depolarization-activated TEA+-sensitive KOR channels (Shabala *et al*., 2006; Shabala and Cuin, 2008)
- At the same time, salinity-induced elevation in cytosolic Ca^{2+} (Tracy *et al*., 2008) will lead to a dramatic raise in ROS level
- Resulting from cytosolic-free concentration of calcium $[Ca^{2+}]_{cyt}$ activation of NADPH oxidase
- Via positive feedback mechanism (Lecourieux *et al*., 2002)
- This will cause an additional K^+ efflux via ROS-activated NSCC channels (Demidchik *et al*., 2002)
- The resultant decrease in the cytosolic K^+ pool may activate caspase-like proteases leading to PCD (Fig. 1)

ROLE OF REACTIVE OXYGEN SPECIES (ROS) IN PCD

Salt stress induces cellular accumulation of ROS which can damage membrane lipids, proteins and nucleic acids (Hernandez *et al*., 2000; Mansour *et al*., 2005; Ben-Amor *et al*., 2007; Eyidogan and Oz, 2007). Vacca *et al*. (2004) also found that ROS increase was an early event of PCD induced by salt in tobacco bright-yellow 2 cells, indicating that oxidative damage and PCD are associated. Lin *et al*. (2006) found that increased ROS was observed before the appearance of characteristics of salt stress-induced PCD in tobacco protoplasts and the addition of ascorbic acid (AsA) significantly decreased ROS levels and the percentage of protoplasts undergoing PCD.

SALT STRESS INDUCED NUCLEAR AND DNA DEGRADATION IN PLANTS

PCD is often associated with the occurrence of specific biochemical and morphological features such as condensation of the nucleus and cytoplasm, fragmentation of genomic DNA (DNA laddering) and fragmentation of the cell into membrane-contained vesicles (apoptotic bodies) (Hoeberichts and Woltering, 2003). One key event in PCD is DNA degradation, because the degradation of the genome is considered to be a means by which the cell death program is made irreversible and facilitates the disassembly of the nucleus. Indeed, DNA degradation is a hallmark of apoptosis during PCD in animal cells (Wyllie, 1980; Jacobson *et al*., 1997).

The nuclear degradation was observed by Katsuhara and Kawasaki (1996) when the roots were exposed to more than 300 mM NaCl for 24 h. Biochemical analysis revealed that nuclear degradation was accompanied by apoptosis-like DNA fragmentation. Katsuhara (1997) investigated the salt stress-induced cell death in barley roots. Cleavage of nuclear DNA was observed 1 h after salt stress. Oligonucleosomal fragments of DNA were detected electrophoretically 8 h after salt stress. These phenomena indicate that apoptosis-like cell death can occur under salt stress. Tao *et al*. (2000) studied the changes in the nuclei of meristematic root cells of soybean in response to severe salinity. TEM observation and agarose gel electrophoretic analysis confirmed that the root tip nuclear DNA deformed or degraded with 150 mM or higher NaCl concentrations.

BIOTECHNOLOGICAL STRATEGIES FOR SALT STRESS TOLERANCE

The basic resources for biotechnology are genetic determinants of salt tolerance and yield stability. Implementation of biotechnology strategies to achieve this goal requires that substantial research effort be focused to on identify salt tolerance effectors and the regulatory components that control these during the stress episode (Hasegawa *et al*., 2000). Further knowledge obtained about these stress tolerance determinants will be additional resource information for the dissection of the plant response to salinity, which will reveal how plants sense salt stress, transducer signals to mediate a defensive response and define the signal pathway outputs or effectors that accomplish the processes required for stress survival and alleviation and steady-state growth in the saline environment.

Molecular genetic and plant transformation advances have made it feasible to assess biotechnological strategies based on activated signal cascades, engineered biosynthetic pathways, targeted gene or protein expression or alteration of the natural stress responsiveness of genes for development of salt tolerant crops (Hasegawa *et al*., 2000; Zhu, 2001). The molecular identities of key ion transport systems that are fundamental to plant salt

tolerance are now known (Hasegawa *et al*., 2000). More recently, the SOS salt stress signaling pathway was determined to have a pivotal regulatory function in salt tolerance, fundamental of which is the control of ion homeostasis (Hasegawa *et al*., 2000; Sanders, 2000; Zhu, 2000).

SALT TOLERANCE USING TRANSGENIC APPROACHES

The over expression of SOS1 improved the salt tolerance of Arabidopsis, demonstrating that improved salt tolerance can be attained by limiting NaC accumulation in plant cells (Shi *et al*., 2003). Similar results were obtained when the of protons generated by the vacuolar HC-translocating enzymes, the HC-ATPase and the HC-PPiase (Blumwald, 1987). The overexpression of AtNHX1, a vacuolar NaC/HC antiporter, in Arabidopsis resulted in transgenic plants that were able to grow in high concentrations of salt (Apse *et al*., 1999). The paramount role of NaC compartmentation in plant salt tolerance has been further demonstrated in transgenic tomato plants overexpressing AtNHX1 (Zhang and Blumwald, 2001). The transgenic tomato plants grown in the presence of 200 mM NaCl were able to grow, flower and set fruit. Although the leaves accumulated high concentrations of sodium, the tomato fruits displayed low amounts of sodium (Zhang and Blumwald, 2001). Similar results were obtained with transgenic *Brassica napus* (canola) overexpressing AtNHX1 (Zhang *et al*., 2001).

Sodium accumulated in the leaves of transgenic plants grown in the presence of 200 mM NaCl formed up to 6% of the dry leaf weight, but the seed yields and oil quality were not affected, demonstrating the potential use of this technology for agricultural use in saline soils. Similar results have been reported in other species. The introduction of a vacuolar NaC/HC antiporter from the halophyte *Atriplex gmelini* conferred salt tolerance in rice (Ohta *et al*., 2002). The overexpression of the rice vacuolar NaC/HC antiporter (OsNHX1) in rice also conferred salt tolerance to the transgenic plants (Fukuda *et al*., 2004). Recently, several reports have further demonstrated the importance of vacuolar NaC compartmentation in plant salt tolerance (Wu *et al*., 2004; Yin *et al*., 2004; Xue *et al*., 2004; Wang *et al*., 2004; Lu *et al*., 2005). The overexpression of AtNHX1 resulted in enhanced salt tolerance in transgenic maize (Yin *et al*., 2004) and wheat (Xue *et al*., 2004). The overexpression of BnNHX1 (Brassica napus), HbNHX1 (barley) and GhNHX1 (cotton) resulted in enhanced salt tolerance in transgenic tobacco (Wu *et al*., 2004; Wang *et al*., 2004; Lu *et al*., 2005). Additional evidence supporting the role of vacuolar transport in salt tolerance has been provided by Arabidopsis plants overexpressing a vacuolar HC-PPiase (Gaxiola *et al*., 2001). Transgenic plants overexpressing AVP1, coding for the vacuolar HC-pyrophosphatase, showed enhanced salt tolerance that was correlated with the increased ion content of the plants. These results suggest that the enhanced vacuolar HC-pumping in the transgenic plants provided an additional driving force for vacuolar sodium accumulation via the vacuolar NaC/HC antiporter.

Challenges for Salt Tolerant Plants

The salt tolerance of the plants in the field needs to be evaluated and, more importantly, salt tolerance needs to be evaluated as a function of yield. The evaluation of field performance under salt stress is difficult because of the variability of salt levels in field conditions (Richards, 1983; Daniells *et al*., 2001) and the potential for interactions with other environmental factors, including soil fertility, temperature, light intensity and water loss due to transpiration. Evaluating tolerance is also made more complex because of variation in

sensitivity to salt during the life cycle. For example, in rice, grain yield is much more affected by salinity than is vegetative growth (Khatun and Flowers, 1995).

In tomato, the ability of the plants to germinate under conditions of high salinity is not always correlated with the ability of the plant to grow under salt stress because both are controlled by different mechanisms (Foolad and Lin, 1997), although some genotypes might display similar tolerance at germination and during vegetative growth (Foolad and Chen, 1999). Therefore, the assessment of stress tolerance in the laboratory often has little correlation to tolerance in the field. Although there have been many successes in developing stress-tolerant transgenics in model plants such as tobacco, Arabidopsis or rice (Grover *et al*., 2003), there is an urgent need to test these successes in other crops.

Rice has the advantages of being both the model monocot and an important crop. However, this is not the case when transgenes are tested with tobacco or Arabidopsis (Grover *et al*., 2003). There are several technical and financial challenges associated with transforming many of the crop plants, particularly the monocots. First, transformation of any monocot other than rice is still not routine and to develop a series of independent homozygous T2 lines is costly, both in money and time. Second, the stress tolerance screens will need to include a field component because many of the stress tolerance assays used by basic researchers involve using nutrient-rich media (which in some cases include sucrose). This type of screen is unlikely to have a relationship to field performance. Third, because saline soils are often complex and can include NaCl, $CaCl_2$, $CaSO_4$, Na_2SO_4, high boron concentrations and alkaline pH, plants that show particular promise will eventually have to be tested in all these environments. Conventional breeding programs for selecting salt tolerant genotypes have met with limited success. This lack of success is in part because breeders prefer to evaluate their genetic material in ideal conditions. This issue is becoming more urgent because of the growing interest of commercial seed companies in making salt-tolerant crops.

From a business perspective, for plant breeding companies to invest in the development of new varieties with enhanced stress tolerance, there will always be the question of whether investing in the development of these cultivars is worth the effort. There is no benefit in developing salinity tolerant plants unless there are economic drivers that will allow the plant to be competitively productive with non saline- tolerant plants growing on uncompromised soil. The view point of basic researchers might differ from this because, for the researchers, the actual, albeit small, increase in salt tolerance is worth the effort. In evaluating the possibility of improving stress tolerance in plants, there are several elements that should be considered. First, although it has been recognized by many researchers that there are dramatic changes in gene expression associated with all types of stresses, the promoters that are most commonly used for transgene introductions are primarily constitutively expressed, including the CaMV35S promoter, ubiquitin and actin promoters (Grover *et al*., 2003).

Recent studies have noted that the over expression of specific stress-induced genes under the control of stress-induced or tissue-specific promoters often display a better phenotype than the same genes expressed under a constitutive promoter (Zhu *et al*., 1998; Kasuga *et al*., 1999). Second, although there have been several successes in producing abiotic stress-tolerant tobacco and Arabidopsis plants, we now need to begin introducing these tolerance genes into crop plants. Moreover, even though researchers tend to focus on a few basic plant systems, with Arabidopsis, tobacco and rice being the major species of choice, there has been no attempt to choose specific genetic backgrounds. Plant breeders have already developed many genotypes that have been selected for traits such as high

yields, enhanced resistance to pathogens and improved tolerance to abiotic stress. The use of these already selected germplasms for transformation with the different genes identified should be emphasized. It is likely that the effectiveness of a specific transgene will be based on the specific genetic background into which it is transformed. One component of this is the well known phenomena of position effect, but the ability of a transgene to work might well be determined by the overall genetic background, independent of the chromosomal location of the transgene, referred to as the transgene combining ability.

Opportunities

Although, progress in improving stress tolerance has been relatively slow, there are several opportunities and reasons for optimism. Over the past ten years, several functional tools have been developed that have enabled us to dissect many of the fundamental questions associated with stress tolerance. These include: (1) the development of molecular markers for gene mapping and the construction of associated maps, (2) the development of EST libraries, (3) the complete sequencing of plant genomes, including Arabidopsis, rice and maize, (4) the production of T-DNA or transposon-tagged mutagenic populations and (5) the development of several forward genetics tools that can be used in gene function analysis such as TILLING (Colbert *et al*., 2001). Thus, we need to focus on looking at the comparative effects and interaction of specific transgenes within a defined genetic background and determine the efficacy of these approaches in the field.

In addition, we should be aware that the overexpression (or the suppression) of a particular gene not only affects the function of the gene product but also affects different pathways. For example, transcriptional profile analyses of AtNHX1 knockout plants growing in the presence or absence of salt revealed that, in addition to the changes in the expression of the vacuolar NaC/HC antiporter, the expression of genes encoding proteins associated with intravesicular trafficking and trafficking to the nucleus and the Golgi apparatus were also affected. This supports the notion that, in addition to its role in the accumulation of NaC into the vacuole, AtNHX1 plays a significant role in protein trafficking and protein targeting, probably via the regulation of the acidic intravesicular pH (Sottosanto *et al*., 2004). Research on the physiology of salt tolerance has demonstrated that the overall trait is determined by several sub-traits, any of which can in turn be determined by several genes. A combination of genome-wide patterns of expression (DNA arrays) and QTL mapping could provide important information with regards to how the expression of genes associated with the QTL region are affected by a particular environmental or experimental condition. Once particular regions or genes are identified, their over expression in the salt-sensitive lines and/or their silencing in the salt-tolerant lines could provide a further assessment of the physiological role of the gene products and, more importantly, the identification of other regions of the genome interacting with the QTL under study. We believe that by comparing different genes and genetic combinations, researchers will be able to advance the field more quickly and develop salt-tolerant germplasms.

CONCLUSION AND FUTURE PERSPECTIVES

Salinity is a major environmental stress that causes PCD in plant cells and is a substantial constraint to crop production. By reducing the PCD caused by salt stress in plants is very useful for farmers to increase the crop production in salt affected area. The importance of studying and understanding programmed cell death in plants may assist in our ability to manipulate this process and thereby provide a useful tool in agricultural and

post-harvest industries. Most of our understanding of PCD has been obtained from studying animal systems. Fortunately, PCD seems to be a highly conserved process across all eukaryotes and much of what has been identified to occur in animal system has also been observed to take place in the plant kingdom. Plant PCD work is at an infant stage and much more work has to be done to understand the PCD occurs by salt stress.

Transgenic technology will undoubtedly continue to aid the search for the cellular mechanisms that underlie tolerance, but the complexity of the trait is likely to mean that the road to engineering such tolerance into sensitive species will be long. Current efforts to produce salt-tolerant conventional crops are aimed mainly at increasing the salt-exclusion capacity of glycophytes. However, these efforts have not produced breakthroughs in salt tolerance, as was predicted twenty years ago. Research with halophytes, by contrast, have identified several prospective crop species and have demonstrated the overall feasibility of high-salinity agriculture, given suitable germplasm. Progress in producing highly tolerant cultivars of conventional crops may require a change in strategy, to attempt to introduce halophyte genes directly into glycophytes. Such research has not even begun, but the tools are at least being assembled, including an understanding of the molecular determinants of salt tolerant plants. In the meantime, the quickest way forward may be the direct domestication of halophytes, which have already been used to demonstrate the feasibility of high-salinity agriculture.

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