Salt and Crops: Salinity Tolerance

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Soils are delicate structures, and careless agricultural practices can destroy them. Crop decline resulting from increased soil salinity can be traced back thousands of years to Sumer and Babylonia. There, the original "Fertile Crescent" eventually turned into desert, destroyed by deforestation, unwise planting, and irrigation-induced soil salinity. Such decline in soil productivity also was evident in the U.S. Dust Bowl of the 1930s and is occurring in present-day Australia.

Irrigation, currently practiced on about 15% of the world's farmland, produces more than one-third of the world's crops but often leaves soils with high salinity. Increased demand for fresh water for urban use not only decreases the amount available for agriculture but also requires that less suitable, often moderately saline, water serves as a replacement. In addition, replacing perennial native plants, which evaporate most of the rainwater, with crops that grow only part of the year can lead to a rise of saline groundwater that ultimately will kill many plants (Figure 1). Flooding, incursion of sea water into coastal fresh water reservoirs, and erratic weather patterns, which seem to increase and which add drought years, compound the effects of increasing soil salinity.

The problem for crops in soils with elevated salinity, contributed by a number of metal ions, is that irrigation without connection to the ground water table inevitably and over time increases the concentration of ions in the root zone. High ion concentrations in the soil affect water uptake and transpiration; the equally inevitable uptake of these ions into the plant body causes ionic and electric imbalances, less growth, and delayed development, and may lead to senescence and plant death. Most deleterious is an abundance of sodium ions, which many plants tend to confuse with potassium. Potassium is essential for a plant's functioning, whereas the sodium ion represents a nuisance that most extant plants, especially those converted into crops, have not experienced during millions of years of evolution. More than 20 years ago, calls urging that research should be directed to this problem went largely unheard but now have inspired another generation of research scientists.

Advances became possible with the advent of molecular genetics—the detection of an increasing number of genes that determine plant behavior in highly saline conditions—and the manipulation of genes by engineering. The breakthrough, however, had to wait for yet another technology. It started with the consideration that in randomly mutated plant populations certain individuals might be found that were less able to tolerate salinity than their parents, and that the underlying genes required for tolerance needed to be understood.

The Salt-Overly-Sensitive Pathway Story

The scientific investigation of salinity stress tolerance from a genetic point of view stumbled along for many years. In part, researchers may have concluded that salinity tolerance as a multigenic trait would make the breeding of crops to grow and produce in saline environments an extremely difficult task. All of this changed and many views about

the genetic basis of salinity tolerance in plants were turned upside-down as a result of the pioneering work of Jian-Kang Zhu and colleagues on a tiny weed called Arabidopsis. Early efforts to dissect the genetic basis of salt tolerance met with considerable skepticism because Arabidopsis is not particularly salt tolerant. Many researchers argued that it would not be possible to find salt tolerance genes in a salt-sensitive plant. But that concern didn't come to pass. The first gene essential for a plant's ability to survive and grow in the presence of sodium chloride (NaCl) was called SOS3 for Salt-Overly-Sensitive-3. It turned out that even though Arabidopsis was not very tolerant, it could be significantly less tolerant if it did not have a functional SOS3 gene.

As a result of genetic screening in Arabidopsis, researchers at the Zhu lab and others have been able to identify many other SOS genes controlling salt tolerance, which has extended the picture of the genetic basis of salt tolerance in plants in general. In fact, some genes have been shown to affect salt tolerance in transgenic plant experiments directly. Increased salt tolerance was reported in plants overexpressing the NHX1 gene that encodes a **[Au: Please spell out Na$^+$/H$^+$ and explain entire term that follows.]** vacuolar Na$^+$/H$^+$-antiporter protein, and by the use of other genes that are less intuitively associated with salinity.

Prominent among these new molecular genetic studies and discoveries, however, is the SOS pathway and its contributions to understanding of the physiological, biochemical, and genetic basis of salt tolerance. SOS3, the first gene in the SOS pathway, encodes a calcium-binding protein that becomes activated by calcium. It had been known that NaCl exposure causes an increase in **[Au: Please spell out Ca^{++} at first use.)** Ca^{++} inside the cell, but before the discovery of SOS3 it was not known how increased Ca^{++} could be connected to NaCl tolerance. After SOS3, SOS2, which encodes a protein kinase activated by SOS3 (but only after SOS3 is activated by Ca^{++}), was discovered. SOS3 also functions to recruit SOS2 to the cell membrane. At the plasma membrane,

activated SOS2 modifies SOS1, a third member of the pathway. SOS1 encodes a Na^+/H^+ antiporter protein that functions to expel Na^+ ions out of the cell in exchange for H^+ ions (Figure 2). This process, Na^+ efflux, may well be the most crucial process needed to establish NaCl tolerance in plants. Transgenic plants overexpressing SOS1 should show tolerance (Figure 3).

But the SOS pathway story continues. SOS1 may be more than a **[Au: OK? Use "a Na$^+$" on p. 3.)** Na^+ efflux protein. Evidence gathered by the Zhu and Pardo labs established SOS1 as an important regulator of other processes involved in salt tolerance. Recent evidence collected by these labs suggests that there exists, at least in part, a controlling effect of SOS1 on how plants respond to stress injury. In fact, SOS1 may even be the sensor that detects the presence of toxic levels of Na^+ inside the cell. SOS1 appears to be the alpha and the omega, the beginning and the end of the SOS pathway controlling Na^+ efflux as well as other processes controlling salt tolerance.

Because SOS1 plays such a pivotal role in controlling salt tolerance, much effort has been directed toward identifying more effective forms. The Pardo and Zhu labs have created and tested mutant forms of the original SOS1 Arabidopsis gene using bakers yeast as a screening machine to detect more active SOS1 proteins. This process has led to superactive SOS1 proteins that confer hypertolerance to NaCl.   Mutant alleles have increased the salt tolerance of yeast an impressive 20-fold, allowing growth in media supplemented with up to 1 **[Au: Please define "M" unit.]** M Na^+ (Figure 4). These novel alleles of SOS1 developed in the laboratory currently are being tested in plants.

The Quest for Even Better Halotolerance Genes

Although Arabidopsis SOS can impart *halotolerance* (the adaptation of living organisms to conditions of high salinity), the gene comes from a plant that is salt-

sensitive to begin with, a "glycophyte." Are there better versions (alleles) in nature that would result in even greater halotolerance? One obvious place to search for such genetic variants is *halophytes* (plants that naturally grow where they are affected by salinity in the root area), which may carry SOS1 homologs [**Au: Please define and/or explain "homologs"]** better suited for the task. SOS1 alleles now are being isolated from an array of halotolerant species that will be tested functionally in transgenic plants. Among these species is *Thellungiella halophila*, a close relative of *Arabidopsis thaliana,* which is extremely tolerant to salt and cold.

Ongoing research holds promise to identify and implement a solution to the problem of soil salinity and crops that has plagued agriculture from its very beginnings. Engineering plants to grow in the sea is not the primary goal of this research. Rather, using modern molecular genetic tools, scientists will be able to develop crops that can grow, mature, and set seed in an agriculturally sustainable way in places where the land previously could not sustain the families who are dependent on it for their food.

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Textbox 1. The ancient and modern scourge of salinity

"The whole land will be a burning waste of salt and sulphur. Nothing planted, nothing sprouting, no vegetation growing on it." (*The Bible*, Deuteronomy 29:23)

5000 B.C.   Irrigation agriculture began in "The Fertile Crescent" of

Mesopotamia (southern Iraq).

2300 B.C.	In the Indus River Valley of India, another civilization developed based on irrigation farming.
1900 B.C.	Extensive irrigation caused soil salinization; as a result, cities along the Indus River depopulated.
1800 B.C.	Soil salinization of farmlands forced Sumerians to switch from wheat to barley (which tolerates salinity better), but steadily increasing salinization eventually destroyed agriculture. The world's first civilization collapsed.
1970 A.D.	The construction of the Aswan Dam in Egypt raised the water table, and salts from the groundwater were drafted by capillarity to the surface of the soil, causing salinization of arable land.
2005 A.D.	About 20% of all irrigated farmland in the world, 23% in the United States alone, is affected by salinization. At the current rate, soil salinity is destroying at least 3 hectares of arable land every minute (FAO 2000).

Figure 1. Australian landscape showing saline groundwater reaching the surface, a result of the removal of native vegetation.
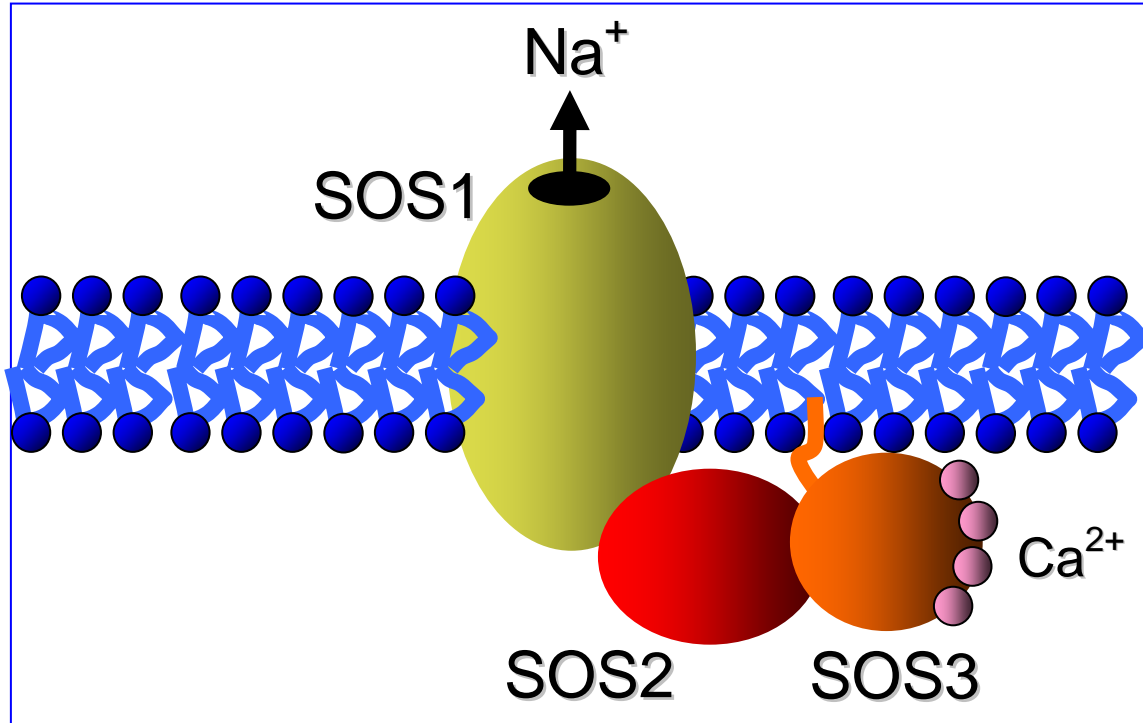
Figure 2. A scheme of the SOS pathway. The membrane-anchored, calcium-activated SOS3 protein directs the SOS2 protein kinase to interact with and activate the SOS1 antiporter.

Figure 3. Arabidopsis plants that synthesize more of the SOS1 protein are protected against high concentrations of NaCl. Top panel shows increased tolerance by SOS1 overexpression. Front row are wild-type controls. The lower panel shows increased sensitivity of SOS1 mutants (lower row).
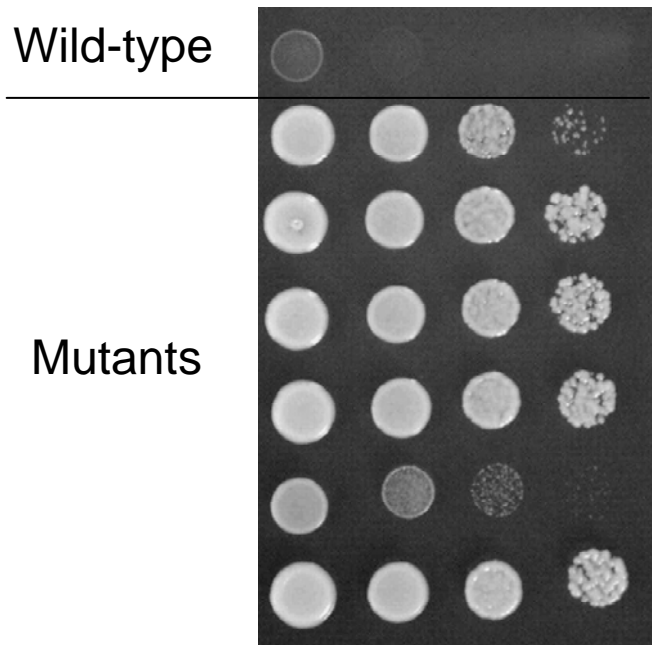
Figure 4. Mutant forms of SOS1 tested in yeast cells. The top row shows the protective

effect of the wild-type SOS1 protein on yeast cells grown on high NaCl. Below are six

different mutated SOS1 proteins that protect the yeast cells to a significantly larger extent.

Yeast cells are diluted successively from left to right, indicating that a ~20-fold higher

protection or efficiency has been accomplished with some of the mutated proteins.

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