

Genetic engineering of salinity-tolerant plants

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Molecular techniques such as recombinant DNA technology may ultimately have their most significant effect on agriculture. Recent advances raise the possibility of the development of new plant germplasm through the introduction of any gene from any organism into plants. Several leading laboratories have achieved the transfer and expression of bacterial and foreign plant genes in plant cells. Increasing attention is now being paid to the use of recombinant DNA technology to isolate and transfer genes governing agriculturally important characteristics such as salinity and drought resistance.

Plant geneticists in this field hope to develop new sources of stress-resistant germplasm for crops. The ultimate goal of producing stress-tolerant plants is so challenging that, in practice, an approach fully integrating molecular and other methods is most likely to succeed (fig. 1).

The identification of salinity-tolerance genes, which can be transferred into plants using the new generations of vectors now being developed, is a critical aspect of the new molecular technology (fig. 2). Manipulation of salinity-tolerance genes using genetic engineering technology requires, first, a clear view of the physiological and biochemical steps involved in cellular adaptation to osmotic stress and, second, thorough knowledge of the *osm* gene or genes governing osmotic tolerance.

The gene/system

Many organisms use a simple rule of chemistry to live in a world deficient in available water. They have evolved sophisticated mechanisms for balancing their osmotic strength (inside) with that of their surroundings (outside): they are able to

avoid dehydration by taking up or synthesizing molecules that work as osmotic balancing agents. Bacterial studies have recently shed light on the mechanisms of cellular adaptation to osmotic stress. Of approximately 150 metabolites tested so far in bacteria, only the betaine series (amino acid derivatives often found in seeds) have possessed potent biological activity in promoting growth under strongly inhibitory levels of osmotic strength. The most active molecules include glycine betaine and its precursors, proline betaine; free proline is also active but under lower levels of stress. Trimethyl- γ -aminobutyrate is also active.

Plants have evolved a variety of mechanisms for adapting to osmotic stress, one of which is cellular adaptation, or osmoregulation. Several researchers have hypothesized that the same class of osmoprotective molecules that work in bacteria (glycine betaine, proline betaine, proline, etc.) and are found in scores of higher plants, also behave as osmoprotectants for plants. These molecules are thought to accumulate in plant cells during osmotic stress and to prevent damage from cellular dehydration by balancing the osmotic strength of the cytoplasm with that of the environment. Evidence is now available that the increase in glycine betaine or proline betaine accumulation in a wide array of higher plants is correlated with the increase in osmotic strength of the environment caused by sodium chloride, and the like.

The metabolism of the apparently osmoprotective molecules in plants has been studied in some detail. Accumulation of osmoprotectants against stress would require the use of fixed carbon and nitrogen — both valuable plant resources. However, several workers have proposed that this cost to the plant may be minimized by localization of osmoprotectants in key regions of the plant cell.

Some encouraging signs are coming from studies in which osmoprotection has been achieved at the whole-plant level by addition of these compounds directly to the nutrient solution. For example, the French researcher, D. Rudulier, who has made several important contributions to this field, recently reported preliminary experiments in which symbiotic nitrogen fixation by nodulated alfalfa seedlings was protected by addition of glycine betaine or proline betaine; in this case, stressed plants (11,600 mg/L sodium chloride) lost essentially all of their nitrogen-fixation capacity, whereas plants treated with 10 mM (1,350 mg/L) betaines recovered up to 30 percent of their capacity when compared with the untreated controls. These workers have also reported that the symbiotic root-nodule bacterium for alfalfa, *Rhizobium meliloti*, is protected in the free-living state by these same molecules. Protection at the whole-plant level requires the addition of relatively high levels of betaines to the nutrient solution, a situation unlikely to occur in nature. For this reason, we have proposed insertion of recombinant plasmids in rhizobia that overproduce osmoprotectants within the nodule itself.

Any gene

The genes governing production of osmoprotective molecules — *osm* genes — are present in plants but have not yet been studied in detail from this source. It is more convenient to study these genes in bacteria.

It is now possible to construct osmotic-tolerant bacteria. For example, in work by Rudulier and co-workers in the Plant Growth Laboratory at UC Davis, a nitrogen-fixing bacterium, normally extremely sensitive to osmotic stress, was converted to a more hardy form by introduction of an osmotic-tolerance

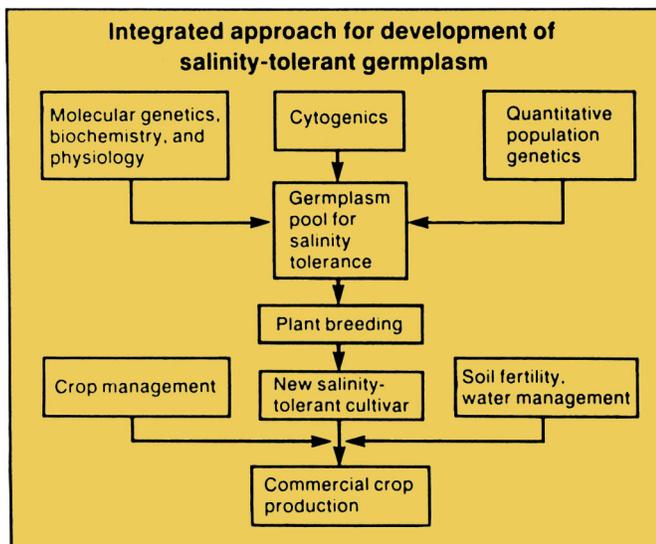


Fig. 1. Integrating molecular and other approaches to breeding may be the best way to develop stress-tolerant plants.

gene. This experiment provided the proof that it is possible to construct stress-hardy microorganisms.

Many recent technological breakthroughs in genetic engineering have involved plasmids, small circular DNA molecules that behave as tiny independent chromosomes. Recombinant DNA techniques have been used to insert virtually every imaginable gene into plasmids, including the stress-tolerance gene. We have chosen to exploit a group of plasmids with an unusual property — the ability to multiply in a wide number of bacterial hosts including symbiotic root-nodule bacteria. A broad-host-range plasmid was chosen that is capable of shuttling back and forth between the bacterium *Escherichia coli* and root-nodule bacteria. In essence, the *osm* genes conferring stress tolerance have been spliced into a broad-host-range plasmid. We have found the *osm* gene(s) in this case to be carried as a segment of DNA of approximately 10,000 base pairs.

This recombinant plasmid, as well as other families of hybrid plasmids that we have recently constructed carrying this same piece of DNA, has at least two properties worth mentioning: (1) the segment codes for the enzymes catalyzing the first two steps in the proline pathway, and (2) biochemical analysis of the first enzyme of this pathway (γ -glutamyl kinase) isolated from cells carrying this plasmid reveals that the enzyme has lost its sensitivity to feedback inhibition by proline. This second property accounts for proline overproduction and osmotic tolerance. To pinpoint the region governing osmotic tolerance, it will be necessary to carry out a DNA sequence analysis of the mutated gene, which should also yield structural information essential for linking this gene to various expression vectors for plants.

Novel symbiotic vector

A new vector system for genetic engineering of stress-tolerant plants is being developed by Dr. J. Kjosbakken and A. Dandekar in the Plant Growth Laboratory. Gall-forming bacteria (such as *Agrobacterium tumefaciens*) and root-nodule bacteria (rhizobia) are potential vectors for delivering useful compounds such as the *osm* gene to leguminous plants (fig. 2). Evolutionarily speaking, these vectors belong to the same tribe of bacteria. They work differently, however: the agrobacterial system delivers foreign genes to host cells, whereas the rhizobial system does not transfer DNA to the host itself but functions as a chemical factory converting raw materials into useful products that are passed on to the host plant (such as fixed nitrogen for plant growth).

The time required for genetic engineering of drought- and salinity-tolerant plants might be shortened by harnessing root nodule bacteria that have already evolved a highly effective symbiosis with leguminous plants. Indeed, the root nodules of a soybean plant may harbor tens of billions of symbiotic bacteria (bacteroids) each working for the host plant. We propose to harness a small portion of their biosynthetic capacity to produce a supply of osmoprotective compounds for protecting the sensitive nitrogen-fixation machinery of bacteroids, nodule tissue, and perhaps even the whole plant against stress. Proline is receiving most of the attention as an osmoprotectant, since raw materials for its synthesis are produced in large amounts in bacteroids, details of this gene are already known, and recombinant DNA plasmids capable of transfer and replication in root nodule bacteria have been constructed.

A number of scientific hurdles remain. For example, the regulatory elements necessary for foreign gene expression (*osm* genes are originally from *E. coli*) may have to be exchanged with regulatory DNA sequences that work in the symbiotic state. Researchers in the Plant Growth Laboratory have

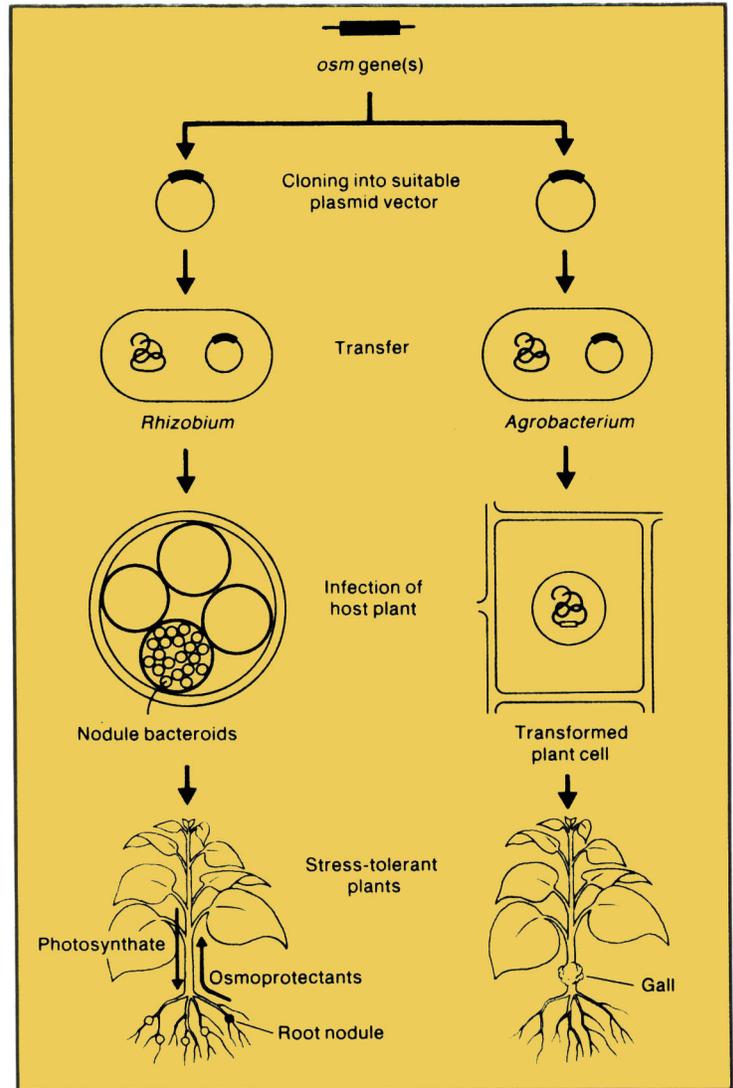


Fig. 2. Two systems by which bacteria may be used to deliver stress-tolerance gene to leguminous plants.

recently made progress on this problem by identifying a segment of DNA that may permit high-level synthesis of foreign genes in root-nodule bacteroids. In addition to *osm* genes, other foreign genes for enhancing the energy efficiency of nitrogen fixation, delivery of plant growth regulators, and perhaps even pest resistance might be expressed by this system.

Conclusion

Cellular adaptation to salinity (osmoregulation) is a fertile area for basic research in plant science. The concept of osmosensory or turgor-sensing proteins linking environmental changes in salinity to dynamic changes in membrane, biochemical, and genetic activities of the cell is one such topic of interest to molecular biologists and biochemists. The discovery of osmoprotective molecules and new classes of *osm* genes may lead to applications such as genetic enhancement of salinity and drought tolerance in crop plants. To achieve this goal, the emerging technology must be closely integrated with established procedures of plant improvement.

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