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A review of genetic advances on breeding salt-tolerant crops

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Large areas of land throughout the world cannot be used for food production because of the limitations imposed by natural or man-made environmental stresses. Therefore, the study of stress phenomena and related tolerance mechanisms is becoming more important. A stress, as defined by Blum (1980), is any environmental factor capable of reducing the yield below the potential level, i.e., the highest possible yield for a given set of conditions. An efficient strategy for improving plants to be adapted to extreme environments would maximize the use of cultivated land and increase the utilization of marginal soils. Because of the economic impact of stresses and the large amount of energy required to alter the environment to suit the plant, it is becoming increasingly important to utilize existing techniques to breed plants better adapted to stress.

The purpose of this review is to summarize the advances and prospects for the genetic improvement of salt tolerance in cultivated crops. The genetic approach to the salinity problem is fairly new, but has generated considerable interest worldwide. This approach provides exciting prospects for increasing productivity in salt-affected areas.

I - Salt stress mechanisms

According to Stavarek and Rains (1984), plants exposed to saline environments encounter three basic problems:

1. A reduction in water potential of the surrounding environment results in water becoming less available.

2. Toxic ions can interfere with the physical and biochemical processes of the organism.

3. Required nutrient ions must be obtained despite the predominance of other ions.

Different strategies have been adopted by plants to overcome the difficulties of water stress. According to Jones (1987), plants avoid salt stress through osmotic adjustment. This may be achieved primarily by:

1. Restriction of inorganic ion uptake and the biosynthesis of organic solutes.

2. Absorption of inorganic solutes from the soil environment where features minimizing ion toxicities must be brought into play.

This raises the question of whether selection should be made for ion exclusion or accumulation. In part this will be dependent upon the availability of genetic resources for either mechanism. The mechanism of salt exclusion has been one of the most frequently reported to differentiate between salt-sensitive and tolerant crop cultivars (Shannon, 1982).

The multitude of specific ways in which plants can potentially cope with salinity has important implications for developing tolerance in sensitive

crops. The more successful cultivars will likely draw upon more than one mechanism. The plant breeder can identify the most useful genotypes for salt tolerant traits and construct appropriate genetic material.

Cellular adaptation to osmotic stress is a cardinal biological process that protects organisms against the lethal effect of dehydration. Bacterial studies (Le Rudulier *et al.*, 1984) have recently shed some light on osmoregulation. 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 include glycine betaine and its precursors, proline betaine, free proline and trimethyl –ϒ– aminobutyrate. These metabolites are controlled by a next class of genes identified by Le Rudulier as Osm genes that protect *E. coli* against osmotic stress. Several researchers (Valentine, 1984) have hypothesized that the same class of osmoprotective molecules that work in bacteria and are found in many 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.

II - Strategies in osmoregulation research

According to Moore (1984), there are three major research strategies in developing plants with increased tolerance to saline conditions, although they are not mutually exclusive or exhaustive:

i) Plant breeding from existing gene pools.

ii) Cell culture with subsequent plant breeding.

iii) Genetic engineering.

1. Plant breeding from existing gene pools

This approach involves utilizing existing genotypes, including wild species, and subjecting them to a highly saline environment. Plants that survive and produce economic fields are considered tolerant and are used in further breeding work to develop varieties acceptable for cultivation on a commercial scale (Ramage, 1980). This approach has one negative factor: the potential increase in salt tolerance in a species is limited by the variability of the existing gene pool.

According to Jones (1987), improvement in plants require the presence of genetic variability and the expression of this variability through the phenotype. The most common approach to identify sources of variability for breeding for salt tolerance has been looking among primitive cultivars, landraces, wild species, and world collections for those which exhibit characteristics for salt tolerance.

The wild progenitors have been, and will continue to be, successfully exploited by plant breeders as sources of useful genes for crop improvement. This includes exotic genera such as *Secale* and *Agropyron* (which are remote relatives of wheat) and *Triticum*. Screening for potential genes also holds true for interspecific variability. High salt tolerance at late vegetative stages has been reported among the wild species of tomatoes, *Lycopersicon pennelli* and *L. peruvianum* (Tal and Shannon, 1984). The same is true of *Hordeum jubatum*, a species which tolerates high levels of salt compared to the cultivated barley, *H. vulgare* (Orton, 1980). Kochba *et al.* (1982) that *Citrus auranticum* is more tolerant to sodium chloride than the related species *Citrus sinensis*.

Resourceful as it may appear as a means for extending the limits of genetic variability for salt tolerance, hybridization with exotics is not without substantial obstacles. Barriers may obstruct the initial cross, the hybrid may suffer sterility problems and distorted segregation may further interfere with selection. Moreover, the material as a salt-tolerant variety is usuallly deficient in economical value. The problem of this technique is not the introduction of the genes of interest into a more desirable genetic background, but rather the difficulties of eliminating the great bulk of undesirable genes introduced from the wild donor (Jones and Qualset, 1984).

However, screening for potential genes is not restricted for interspecific variability. Screening for intraspecific variability is also important. In tomatoes, Jones (1987), Jones *et al.* (1987) and Jashin *et al.* (1986) found out that the varieties P1174263 and Edkawy demonstrated superior seedling growth under salt stress. The former also demonstrated high salt germinability and the

latter high tolerance at the late vegetative stage. Both accessions belong to the same cultivated species *L. esculentum*, and both are from arid lands: Edkawy, a local cultivar from the Edko area along the north coast of Egypt, and P1174263 from Turkey. Moreover, Jones (1987) concluded that since stress responses involve essentially all aspects of plant growth, multigenic control for salt tolerance is expected. Genetic interpretations are complicated because of the interaction between life-cycle stages and stress intensities.

The majority of plant breeders working on biosaline problems have treated salinity stress as Na C1 or mixed with CaC12. While convenient, such artificial formulations do not reflect the major ion compositions of naturally occuring saline soils (Epstein and Rains, 1986). Expectations that a line selected for tolerance to Na C1 alone will exhibit equal performance under ionically more complex saline conditions are unreasonable. Jones (1987) believes that considerations must be given to breed for production in a specific saline environment, mimicking this environment. In any given environment, the concentration of soluble salts changes temporally and spatially. Sources of irrigation water are also likely to change in their quality during the course of the growing season. These represent important variables that must be monitored and assessed for the development of an appropriate breeding strategy.

2. Cell culture techniques

Plant cell culture is one of the methods scientists have for studying how plants tolerate stress and for producing and selecting genetically superior plants. This technique is often praised as a means to perform selections in several petri dishes which would take hundreds of acres if performed at the whole plant level. Moreover, in this approach, cells can be subjected to mutagenic agents in order to expand the variability of the gene pool beyond that available in nature. Advantages and disadvantages are given by Rains (1981). This includes increased control of environmental factors, a greatly expanded number of treatments and replications with reduced manpower, and a vastly increased potential for selection of salt-tolerant variants. Disadvantages includes the difficulty of selecting for characteristics that are manifested in subsequent growth stages such as yields. Thus the cells must be regenerated and grown out, thereby providing additional breeding material for standard breeding and selection work.

For several years, scientists have been reconsidering the concept of tissue culture as a neutral pathway from which to produce copies of an initial genotype. The unexpectedly high recovery of new phenotypes in cell culture without deliberate mutagenesis has been puzzling. The variation among plants derived from cell culture was given the name **somaclonal variation** by Larkin and Scowcroft in 1981. They reviewed somaclonal variation in sugar cane, potato, tobacco, rice, oats, maize, barley and *Brassica* species. Larkin and Scowcroft suggested a number of possible genetic mechanisms, many or all of which may prove to contribute to somaclonal variation. Among these are: chromosome re-arrangements, transposable elements, somatic gene re-arrangements, gene applification and depletion and re-arrangements in organelle genomes.

The application of cell culture technique to breed plants to stresses is relatively recent. Zenk (1974) reported the isolation of a resistant cell line from haploid cells in *Nicotiana sylvestris*. At 1% Na C1, the resistant cells grow at 50%, the rate of that of the control without Na C1. No growth occurred at 1% Na C1 for the nonselected cells. Nabors *et al.* (1975) obtained *N. tabacum* cells which showed tolerance to 0.16% Na C1 and then to 0.52%. Salt stress was applied stepwise to the suspension culture.

Later, Nabors *et al.* (1980) produced lines tolerant to 0.88% Na C1. Dix and Street (1975) were able to select cell lines of *N. sylvestris* and *Capsicum annuum* resistant to 1% and 2% (W/V) Na C1. The cells were selected in suspensions which contained Na C1. After several passages, they were able to obtain cell lines whose growth equalled or exceeded the control. Croughan *et al.* (1978) isolated Na C1-resistant cells which could grow on a medium containing about 1% Na C1 from a cell culture of *Medicago sativa*. The selected line behaved like a halophyte in some respects, including the need for salt for optimal growth.

Rains *et al.* (1980) selected cells of rice in the presence of about 2 – 3% Na C1a concentration lethal for the unselected cells. The selected cells required the presence of sodium chloride for optimal growth. Kochba *et al.* (1980) reported the isolation of orange callus lines with increased resistance to Na C1. The resistance was

maintained in embryos obtained from these lines. Hasegawa *et al.* (1980) reported the selection of *N. tabacum* cells tolerant to 1% Na C1. These cells, however, lost their tolerance once they had been grown away from salt for five cell cycles.

Kochba and co-workers (1982) recently reported cell lines of *Citrus simensis* and *C. auranticum* tolerant to Na C1. The cells were selected on 0.5% and 0.7% Na C1, respectively, and were able to grow on Na C1 up to 1%. Thomas *et al.* (1982) demonstrated somaclonal variability among ten different plants of potatoes regenerated from these same initial cell line. They indicated that the variability either arose during culture or it pre-existed in somatic cells outside the germ line. If substantial numbers of somatic cells have genomes differing from those destined to form gametes, then the cell culturist does not start with just one genotype in a petri dish. Instead, the petri dish is more comparable to a plant breeder's field of heterozygous plants than previously suspected.

The salt used in the majority of salinity studies is Na C1. Several researchers, however, have compared the response of callus to different types of salts. Golner *et al.* (1977) using *Daucus carota* and Chen *et al.* (1980) using *Nicotiana tabacum* found different responses when they compared growth on seawater, synthetic seawater, manitol, Na C1, and other C^{1-} and SO^{2-} salts. They suggested the use of multiple salts or synthetic seawater as a selection pressure to better parallel the salinity under fields conditions. Kochba *et al.* (1982) tested their salt-tolerant *Citrus* callus cultures which were selected on Na C1 or other salts. The cells were sensitive to K C1 but able to grow well on $Na_2 SO_4$, K_2SO_4 and $MgSO_4$. Stavarek and Rains (1982) compared the Na C1-selected alfalfa cells on different salts (Na C1, KC1, $Na_2 SO_4$ and K_2SO_4. The growth of the Na C1-selected alfalfa line was inhibited at high levels of all salts except Na C1. The non-selected alfalfa cells, however, could not tolerate salinity stress. Stavarek and Rains concluded that the Na C1-selected cells could tolerate Na C1 stress but were sensitive to high levels of K^+ and SO^{2-}. Moreover, when the same growth studies were performed on the Na C1-selected rice cells, differents results were obtained. The Na C1-selected rice cells can tolerate high levels of all salts tested. The non-selected cells did not grow on any salts tested. Stavarek and Rains concluded that the Na C1-selected rice cells have adapted a different mechanism for dealing with growth in a saline environment than the Na C1-selected alfalfa cells.

Hanson (1984) posed the question of the possible types of genetic and chromosomal variation mechanisms (mentioned above) which are likely to be most relevant for salt tolerant breeding. Chromosomal loss, addition, or gross chromosmal re-arrangements are likely to be the least useful, since such changes are often detrimental and may not be transmitted stably through either vegetative or sexual propagation. However, Hanson believes that alien chromosomal breakage and exchange would be of interest in cell culturing interspecific hybrids. Orton and Steidl (1980) have some interesting evidence of such occurrences in hybrids between *Hordeum vulgare* and *H. jubatum*. The latter is a salt tolerant species. Some regenerants were obtained in which all *jubatum* chromosomes have been eliminated. However, some genes are still present which manifest themselves as isozyme bands. Moreover, Hanson (1984) stated that culture induced alterations in smaller units of genetic material, such as could ensue from mobilization of transposable elements, somatic gene re-arrangement, or gene amplification or depletion, could also affect a cell's line response to salt stress. Such changes, however, must be carried to the whole plant level and be stable following propagation for such variation to be useful.

From the above-mentioned discussion, it is clear that in cell culture, genetic diversity can be generated from somaclonal variation. However, this is not the only pathway to create diversity *in vitro*. **Fusion of protoplasts** to overcome species barriers is another achievement of cell culture technique which has potential to be utilized for salt-tolerance plant breeding. Some cell hybrids are produced by isolating and fusing protoplasts from two different species, followed by culture and regeneration to whole plants. With moderate effort, production of protoplasts and their fusion are now possible for a wide range of different plant species (Carlson *et al.*, 1975). Currently, somatic hybrids have been produced in a number of genera, primarily among the *Solanaceae*. Regenerated hybrid plants are generally confined to the same genus, for example between *Petunia parodii* and *P. parviflora* (Power *et al.*, 1980). More examples are cited by Kurmbiegel and Schieder (1979) who selected somatic hybrids after fusion of protoplasts from *Datura innoxia* and *Atropa belladonna*. The well known hybrid between

tomatoes and potatoes may also be cited as a reference (Melchers, 1980).

Protoplast fusion can also result in hybrids containing new combinations of nuclear and organelle genes; from mitochondria and chlorolplasts. Fusion of two somatic cells circumvents the maternal inheritance following sexual hybridization (Billiard *et al.*, 1979). Genes for important productive characteristics such as hybrid vigor and male sterility have been proposed to be located in extra-nuclear organelle. Whether the ability to create new nuclear organelles combinations and novel mitochondrial genomes is useful for salt tolerant genotypes is presently unknown. Investigations of this point may be worthwhile because organelle genomes can specify resistance to such stresses as herbicides (Arntzen *et al.*, 1979).

Stavarek and Rains (1984) drew attention to several problems with cell culture systems that should be taken into consideration.

1. After selection, plants must be regenerated from the selected cells. In most cell culture systems, the capacity for regeneration decreases with time. This, however, may be a technical problem which can be overcome by a better understanding of the processes involved in regeneration. In several species, such as *Daucus carota* (Wochok and Wetherell, 1972) and *Medicago sativa* (Stavarek *et al.*, 1980) plants have been regenerated from relatively old callus. Moreover, regeneration of plants from the salt-tolerant cells has been limited. Kochba *et al.* (1982) obtained embryos from their salt-tolerant citrus cell lines. The embryos grew better in saline media but no production of plants was reported. It is disappointing to state that only a few genotypes, four to six, in both wheat and maize could regenerate into plants from cell culture.

2. The expression of desirable traits in regenerated plants as well as heritability is very important. The plants regenerated should maintain the characteristics of the variety and incorporate the new genetic traits that were selected for in the cells without incorporating any inimical genes. Stavarak *et al.*, (1980) were able to regenerate hundreds of plants from the salt tolerant alfalfa callus once a new media sequence was developed. These plants, which had been in culture for several years, proved to have many lethal genetic changes. The plants were stunted, slow growing and susceptible to many diseases. The amount of tolerance expression in the plants was never fully determined.

Several studies have compared the response of callus to that of the whole plant under increasing salt stress. Positive correlations were found for different species of *Lycopersicon* (Tal *et al.*, 1978) and of *Hordeum* (Orton, 1980b). Smith and Mc Comb (1981a) studied the growth of whole plants and their corresponding callus cultures of salt-sensitive glycophyte *Phaseolus vulgaris*, a salt-tolerant glycophyte *Beta vulgaris*, and two halophytes *Atriplex undulata* and *Suaeda australis* to increasing levels of Na C1 (0 to 250 mM).

For the first species, both the plant and callus decreased in growth with increasing salt. In *Beta*, both callus and plant showed an increase in growth at intermediate salt levels and a decrease at higher levels. For both halophytes, the plants showed increased growth with increasing levels of salt, while the callus growth decreased. Smith and McComb concluded that the halophytes have whole plant mechanisms (salt glands, succulence) for dealing with salts, so tolerance is not expressed in their callus. *Beta* is suggested to have cellular level mechanism for salt tolerance. The same authors (1981b) have also examined the response to increasing Na C1 of three pasture legumes and the corresponding callus and found out very similar responses and suggested that the use of callus to screen for Na C1 tolerance is a valid system.

3. Genetic engineering

The ability to introduce DNA sequences in different cells and to monitor their expression opens new methods for plant breeding. Proper expression of introduced genes is not the only major barrier to the improvement of crops via recombinant DNA technology. Identification and isolation of genes that specify salt tolerance are requisites for directed genetic engineering of crop plants.

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.

Bacterial studies have recently shed light on the mechanisms of cellular adaptation to osmotic stress. The Osm genes which control the production of osmoprotective molecules have been isolated and manipulated in different species of micro-organisms (Le Rudulier *et al.*, 1984). Moreover, Abd El-Halim and Abd El-Salam, at the National Research Center in Cairo, were recently (1985) able to transfer Osm genes from barley and yeast into *Azotobacter chrococcum*.

At the Plant Growth Laboratory of the University of California, Davis, many recent technological breakthroughs in genetic engineering have evolved plasmids (Valentine, 1984). Plasmids are small circular DNA molecules that behave as tiny independent chromosomes in several prokaryotic and possibly eukaryotic organisms. Recombinant DNA technologies have been used to insert virtually every imaginable gene into plasmids including the stress-tolerance genes. The Osm genes (a segment of DNA approximately 10,000 base pairs) have been spliced into plasmids of a broad-host-range from *E. coli*. These recombinant plasmids have two properties:

i) they code for the enzymes catalyzing the proline pathway; and

ii) the particular enzymes have lost their sensitivity to feedback inhibition.

These together account for proline overproduction and osmotic tolerance. These genetically engineered plasmids may be indirectly introduced to plants via:

a) the gall-forming bacteria *Agrobacterium tumefaciens* or
b) the root-nodule bacteria *Rhizobia*.

Both are potential vectors for delivering Osm genes to leguminous plants.

Another route which is currently developed by private genetic engineering companies such as Plant Genetic Inc., Calgene in Davis, California, and Phytogen in Pasadena, California (personal communications) is the use of electroporation and microinjection. The first technique involves forcing naked DNA molecules carrying Osm genes into recipient cells by applying electrical shocks. The second involves microinjection of Osm genes into the germ line or apical meristem and the production of vegetative or sexual projegy characterized with salt tolerance. These direct techniques could be applied in both leguminous and nonleguminous plants.

Several predictions can be made regarding the near-term developments of recombinant DNA technology for plant genetic engineering. With the availability of halophytes, it is easy to speculate that the potential shift in the response curve to salinity tolerance for some economical crops such as wheat and maize is virtually unlimited.

III - Summary and conclusions

Intensive studies have been made concerning the effect of salinity on growth and development of plants. These studies indicated that plants treated with sodium chloride show symptoms of chlorosis, tip and marginal burn on the leaves, disruption in nutrient translocation, mitotic disturbances as well as metabolic imbalance.

An understanding of salt tolerance in plants is important for an effective approach to the salinity problem. Some crops, such as cotton, barley, sunflower and sugarbeets, can be grown in relatively saline soils. Others, including beans and corn, are sensitive to salinity. It is intriguing to speculate that a sensitive crop might be genetically altered to withstand high levels of salinity. In some species, the diversity of salt resistant genes among cultivars seems quite extensive and conventional breeding technologies are used. In many species with little diversity for salt tolerance, the use of variability existing in wild relatives or the use of tissue culture is promising.

Selection and breeding, including the use of wide crosses, from one point of view, represent the best short term approach to the development of salt tolerant plants. Cell culture technology, from another point of view, offers several advantages. These include a well-defined media, a huge number of cells can be screened and evaluated and mutagenic agents can be applied to increase variability.

Recent advances in cell culture and molecular genetics offer a great potential to expand the options available for plant improvement well beyond those of traditional plant breeding. Some cellular techniques such as embryo and pollen cultures and the efficient use of somaclonal

variability should provide practical results in the short term. Protoplast fusion and organelle hybridization will probably be a longer term process, but greatly expand horizons concerning what is potentially feasible.

Genetic engineering involves determining the mechanisms within the plant cell which control the plant's response to saline environment, then locating the genetic codes that control these mechanisms, and finally transferring the DNA molecules that control this process into the genome of the plant of interest. The major problems, however, center on the lack of knowledge about exactly how the control mechanisms within the plant cell operate. Although there are some agreements with respect to bacteria and yeast, gaining and understanding of complex plants will require further investigation.

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