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Salinity Tolerance

INTRODUCTION

Living organisms contain only a selection of the many types of molecules available in the environment. Many of the commonly found ionic molecules in soils and solutions are, in fact, toxic to living cells at relatively low concentrations. For example, Na^+ is reasonably toxic to both animal and plant cells. Its active exclusion by animal cells is, in evolutionary terms, the *raison d'etre* for the nerve impulse and ultimately the brain.

Arid regions of the world are associated with soils which frequently contain high levels of ionic salts such as NaCl, $NaSO_4$, and $CaCl_2$. These salts are normally contained in soils, but in regions of moderate to high rainfall, they are leached from the soil into the ground water and ultimately the ocean, which is therefore quite salty. A complication in arid regions is that the practice of irrigation, no matter how pure the water source, gradually adds even more salts to the soil [1]. This is particularly true when, as is usually the case, the amount of irrigation is insufficient to leach salts into the ground water.

Wetter regions of the world are associated with acidic soils. Rain water contains dissolved CO_2 which is carbonic acid. Hydrogen ions are readily produced by the dissociation of carbonic acid. The chemical activity of H^+ is such that it displaces other cations from soil particles. The other cations (including such useful molecules as Ca^{++}, Mg^{++}, and NH_4^+) are leached into the ground water leaving the soil acidic and nutrient-poor. In addition, toxic metals such as aluminum, which are in a precipitated form in soil of neutral or alkaline pH, are brought into the soil solution in acidic soils.

Thus, both wet and dry regions of the world have salinity problems. In terms of plant production, salinity can be defined as the excessive concentrations of soluble salts in soils [2]. If the salt concentration is high enough to lower the water potential appreciably (as in NaCl stress) (0.5–1.0 bar), the stress will be called a salt stress. If the salt (acid or base) concentration is not high enough to lower the water potential appreciably (as in the case of aluminum toxicity), the stress will be called an ion stress [3].

It is obvious but important to point out that salt stress in soils varies considerably with soil moisture level. A soil with little salt stress at field capacity moisture will be quite stressful under dry conditions which are still suitable for plant growth. Since soil frequently loses most of its water between episodes of irrigation or rain, a soil salinity can easily increase by an order of magnitude.

It is evident from the number of books and review articles published in the past that soil salinity has attracted the attention of several scientists and government organizations. This interest stems from the following data:

A. Of the world's available land area (14 billion hectares) only around 3.2 billion are arable or potentially arable [4].
B. Approximately 25% of the arable or potentially arable land is arid or semi-arid, while another 25% received enough rainfall to make soil acidity a problem ([4]; calculated from Van Wambeke [5]).
C. Of the 1.0 billion hectares currently cultivated, 14% are irrigated and produce half of the world's food [6]. Prospects for increasing the extent of irrigation are not bright since 80% of the world's readily available fresh water is already used in agriculture [7,8].
D. All irrigation systems have a limited useful lifetime due to the increased rate of soil salinization [1]. The management of drought, salinity, and acidity in soils are all energy-intensive agricultural practices in both developing and developed nations.

The role of the plant breeder has become increasingly important as modern agriculture seeks to produce plants with increased fitness for available environments. The economic benefits of such improvements can be easily illustrated. Rice is commonly thought of as a crop which

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is not troubled by lack of water. Yet all rain-fed rice, which constitutes half of the world's 141,150,000 hectares, is subject to drought during at least part of the growing season [9]. World rice production is approximately 350 million metric tons [10]; and rice sells for $418 per metric ton (calculated from data in the *Wall Street Journal*, 1982). Thus, the total world's rice crop is valued at $146 billion. If a drought-tolerant rice could raise production by 10% on that half of the world's crop which is rain-fed, the dollar value of this increase would be over $7 billion.

In terms of the agricultural effects of NaCl, it is worth briefly considering the economic effects of salinization in a single river basin. The Colorado River and its tributaries drain around 583,000 km² in seven states and Mexico [11]. Salinity of the river at its source in the Colorado Rockies is around 50 ppm. In the early 1970's, salt levels at the Imperial Dam exceeded 1000 ppm and were as high as 1200 ppm in Mexico. Using 1000 ppm as a base, it was calculated that 47% of the salt was natural, 49% was due to irrigated agriculture, and 4% was due to industry and municipal sources [12]. It is interesting to note that the natural salt-loading of the river leaves its salinity well below the 500 ppm EPA drinking water standard and considerably below the 800 ppm level generally recognized as the threshold of severe agricultural damage [12]. At present, calculations for the basin show that an increase in 1 ppm salinity at Imperial Dam causes an average agricultural loss of $827,888 per year in the basin (based on Kleinman and Brown [13]; recalculated for 1984 dollars). Efforts to lower salinity in the river have been partially successful (in 1980, salinity at the Imperial Dam was 755 ppm) but extremely expensive in terms of construction and operation costs as well as legal and administrative costs. Projected total damage figures due to Colorado Basin and worldwide salinity are shown in Table 1. This estimate does not include salinity damage incurred in dryland agriculture.

This example will suffice to summarize lots of data which all show the economic, physical, and human costs related to salinization. In general, salt-tolerant plants can be viewed as valuable additions to our agricultural repertoire because:

A. they will increase the useful lifetime of irrigation systems;
B. they will allow crop production of marginal land too salty for currently available cultivars;
C. to the extent that salt tolerance accompanies drought tolerance, which is often the case, they will allow irrigation water to be more economically utilized.

This review concerns several aspects of salt tolerance with chief concern being given to Na^+ salts. The nature of salt tolerance in plants and the efforts of plant breeders to screen for and increase tolerance are briefly considered. Experiments designed to select for increased salt tolerance through tissue culture techniques are discussed in detail.

PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF SALT STRESS AND SALT TOLERANCE

The use of tissue culture selection and gene transfer techniques to obtain improved cultivars of plants will be retarded by a lack of basic information about plant physiological processes and the genes that control them. It is clear that methodological problems of plant regeneration and of gene isolation, transfer, and control will be solved well in advance of obtaining the basic knowledge needed to use these techniques effectively.

This section contains a brief review of the physiological effects of salt on plants and of the mechanisms of salt tolerance. Readers should consult the listed review articles for more complete information.

Physiological Effects of Salt

The physiological effects of salt on plants are diverse and are not clearly etiologically defined. It seems obvious from a strictly logical cellular view that the ac-

TABLE 1. Annual Agricultural and Total Economic Loss Due to Salinity in the Colorado River Basin (Modified Data of Kleinman and Brown [13]).

Maximum Downstream Salt Level (mg/l)	Type of Damage	Colorado River Basin (U.S. Dollars)	Worldwide (U.S. Dollars)
1000	Agricultural	118,945,760	7,136,745,400
	Total	540,782,760	32,446,965,000
1200	Agricultural	403,260,730	24,195,644,000
	Total	909,465,130	54,567,908,000
1400	Agricultural	1,328,320,000	76,699,199,000
	Total	1,918,891,800	115,133,510,000

1976 data was modified to 1984 dollars by using the annual percent change in summary price indexes (estimated at 5% for 1982 and 1983). Worldwide data was calculated on the assumption that 25% of the world's land is arid or semi-arid and therefore subject to salinity problems and that the Colorado River Basin represents 1/60th of this area. The worldwide projection is underestimated because many river basins subject to salinity damage are more developed and populated than the Colorado basin. Total damage figures include municipal damages of all sorts and depend on population density.

cumulation of salt in the vacuole does not physiologically interfere with plant function, whereas accumulation in the cytoplasm has varied, deleterious effects. The cytoplasm of a tolerant plant can adapt to high salt concentrations or can exclude toxic salts while accumulating or producing other osmotica to prevent osmotic water loss. Non-salt-tolerant plants, then, would be those which cannot restrict cytoplasmic salt buildup or modify cytoplasmic components to accommodate accumulation.

On the basis of growth response to saline conditions, two groups of plants can be distinguished. The halophytes ("salt lovers") are capable of survival (i.e., completion of their life cycle) on salt concentrations exceeding 300 mM [7]. Conversely, glycophytes ("sugar lovers," a.k.a., non-halophytes) cannot complete a life cycle at salt concentrations exceeding 300mM. At low (e.g., 20mM to 200mM) salt concentrations, there is an overlap of growth responses between the two groups [14]. The physiology of salt tolerance in both groups at low and high salt concentrations is briefly discussed below. Readers are referred to Flowers, et al. [7], Greenway and Munns [14], Wainwright [15], Rains [16], Wyn Jones [17], and Munns, et al. [18] for detailed discussions of the physiology of halophytes and non-halophytes.

In many crops, resistance to salinity is greater during seed germination than at emergence and early growth, and later stages of growth and development [19-24]. However, sugarbeet and safflower are most sensitive during germination [25].

The type of photosynthetic carbon fixation is correlated with salt tolerance in some plants. Plants which possess the C-4 pathway of carbon fixation require sodium in trace amounts [26,27]. Marginal response for sodium was shown by *Hordeum vulgare* L. c.v. Pallidum, *Atriplex hortense* L. var. *astrosanguineae* and *Lycopersicon esculentum* Mill c.v. grossi lisse, all of which have C-3 carbon fixation pathways [28].

Salinity at sublethal levels causes growth suppression in non-halophytes; however, there is no direct injury to growing cells [29]. In the majority of cases, salinity retards the growth, but ontogeny follows the normal pattern [29]. Salinity limits on productivity shows a wide range in different plant families: legumes (e.g., peas and beans) are most sensitive and can survive at only the lowest salt concentrations; cereals (e.g., rye, oats, wheat, barley) and sainfoin can withstand medium salt levels; only forage plants and others (e.g., sudan grass, alfalfa, sunflower, sugarbeet, and forage beet) can survive at high levels of salt [3,30]. Certain halotypes, however, require some optimal salinity for growth [7].

Suppression of growth may be attributed to a decreased concentration of cytokinins in leaves and xylem exudates [31-33]. However, this alone could not be suggested as a cause for growth suppression as external application of cytokinins had little effect [34]. It may be necessary to consider salt-related cytokinin effect in concert with other growth-regulating substances.

Isolated tissues like coleoptile sections [35], hypocotyl segments [36], cotyledons [37], and leaf tissues [38] have been used to study salinity effects. Cells showed increased turgor pressure, cell wall synthesis, and cell enlargement in response to salt. NaCl suppressed synthesis of RNA and protein and reduced the number of cells replicating DNA [39]. A similar situation was reported in cell cultures. Lethal concentrations of NaCl prevented cell division; once initiated, one cycle of cell division was completed, but cells failed to initiate another [29]. Apparently, salt prevented the initiation of cell division by limiting some factors required to initiate division [40].

Under saline conditions, the cells of halophytes accumulate Na^+ or Cl^- in excess. Non-halophytic species which exhibit some degree of tolerance avoid ion excess in the cytoplasm [14]. Toxic effects of salinity in non-halophytes can be seen on the plasma membrane or on the cytoplasm after accumulation [14]. Orthophosphate (P_i) concentration and utilization are affected under saline conditions as is the active transport of P_i, glucose, and leucine into root cells [41,42].

A comparison of enzymes, such as amylase and invertase in leaves of salt-tolerant and salt-sensitive plants has shown considerable variation in the effects of salt [43,44]. Acid invertase activity was reduced with salinity in bush beans and maize (salt-sensitive plants) whereas in salt-tolerant barley and sugarbeet activity of invertase did not change under salt stress [44,45]. Amylase (alpha and beta) in beans increased with increasing levels of salt. Unlike beans, salt-tolerant sugarbeet leaves showed no change in the amylase activity [43]. These effects may result from direct or indirect exposure to NaCl depending on the extent of salt accumulation by the cytoplasm.

Direct exposure to salt affects several enzymes adversely. Malate dehydrogenase, aspartate transaminase, glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase isolated from halophytic plants showed NaCl sensitivity like those from salt-sensitive plants [46,47]. Peroxisomal glycolate oxidase and phosphohydrolases appear to be relatively insensitive to NaCl *in vitro* [46, 48,49]. Malate dehydrogenase from a halophyte *Suaeda maritima* has two molecular forms. The high molecular weight form remained more active at higher salt concentrations than its lower molecular weight form [50,51]. While *in vitro* studies of the effects of salt on enzymes are important, they need to be complemented with experiments to determine whether *in vivo* enzymes are exposed to salt. Clearly the salt-sensitive enzymes from halophytes are not exposed. X-ray microanalysis for localization and quantification of ions in plant cells is a technique with many applications in the study of salt stress [52,53]. Nuclear magnetic resonance (NMR) is a technique with newly discovered potential for ion quantification (J. Heyser, Los Alamos National Laboratory, personal communication).

Mechanisms of Salt Tolerance

Halophytes exhibit certain morphological and ana-

tomical features in response to salinity [28,54,55]. These include: increase in leaf succulence; expansion of leaf lamina; changes in the number and size of stomata; thickening of cuticle; extensive development of tyloses; increased lignification; changes in number and diameter of xylem vessels; and presence of salt glands.

Salt glands are efficient devices for the secretion of salt from the plant body [56]. These uni- or multicellular structures occur on the epidermis of stem and leaves of halophytes. Cells comprising the salt glands possess dense cytoplasm, many mitochondria, a large nucleus, and small vacuoles containing electron-dense materials [56,57]. The mechanism of salt transport to salt glands has been reviewed by Lüttge [58].

Liphschitz and Waisel [59] reported the presence of salt glands in several glycophytic species of Gramineae. They also suggested a possible common halphytic ancestor for glycophytes and halophytes beloning to Gramineae.

Halophytes growing in highly saline conditions possess a high internal ion-content, unlike non-halophytes, which respond primarily by salt exclusion [60,61]. Different parts of the plant may accumulate ions differently [14,62-64]. Average halophytes accumulate salts at concentrations equal to or in excess of sea water in their leaves (90% of total Na^+ is present in the shoot and 80% is in leaves only) [65,66]. Glycophytes tend to exclude salt from leaves but may accumulate high levels in their roots and stems [65]. To the extent that leaves take up salts, older leaves accumulate more than younger leaves and buds. Halophytes have similar concentrations in old and young leaves [66]. The reasons for these sorts of differences between halophytes and glycophytes are not known. A hypothesis is that low salt levels interfere with glycophytic leaf metabolism sufficiently to interrupt transpiration and prevent further salt from entering leaves, especially younger leaves (especially Na^+, see Ahmad and Wyn Jones [67]).

As stated earlier, many enzymes surveyed in halophytic plants are not resistant *in vitro* to high salt concentrations (in contrast to enzymes of halophytic bacteria). Compartmentation of salt, within the vacuole but not the cytoplasm, which must logically occur as an avoidance mechanism for sensitive enzymes, is difficult to study with existing technologies, although rapid freezing followed by X-ray microanalysis of these sections would appear to be a promising technique [68,69]. The study of meristematic (non-vacuolated) cells would also provide useful information about ion uptake or exclusion by cytoplasm [17]. It is known that chloroplasts of halophytic species accumulate considerable quantities of ions [70–72]. Limited data summarized by Greenway and Munns [14] suggests that salt may accumulate in the cytoplasm of glycophytes but not in that of halophytes (at least as compared to vacuolar salt concentrations).

Accumulation of several types of organic solutes has been suggested to play a role in osmoregulation because of the cellular compatibility with other macro-molecules (reviewed in Yancey et al. [73]). These solutes are generally produced in plants under salt, drought, or osmotic stress [74-77].

The most important of these organic solutes are sugars, free amino acids, methylamines, and polyhydric alcohols. These solutes, unlike salts, do not inhibit *in vitro* enzymes even at a concentration of 500mM [14]. Accumulation of organic solutes seems to occur in association with reduction in protein and polysaccharide synthesis and subsequent growth inhibition [78]. Whether organic solutes actually protect the plant from salt stress or are merely the result of such stress is still a moot point and requires further research. It is also unclear whether halophytes exclude toxic ions from the cytoplasm and rely on organic solutes for osmotic balance, or whether the ions are admitted to the cytoplasm which is somehow protected by the organic solutes ([79-80] but see Pollard and Wyn Jones [81]).

Free amino acids, such as proline, are important regulators of cellular osmotic pressure [82-89]. The accumulation of proline may account for 30-70% of free amino acids [86,90-92]. Chu et al. [93] and Hanson and Nelsen [94] however, considered that the contribution of proline to osmoregulation is minor.

Organic acids like oxalate [95-98], malic acid (in plants with CAM pathway) [98-100] also ionically balance the sodium. Oxalate is most common among halophytes [54,97].

Another class of organic osmoregulators is the methylamines. One which is studied extensively, glycine betaine, is commonly present in all Chenopodiaceae, the family in which most halophytic plants are found [92]. Among Gramineae, only certain tribes possessed glycine betaine [101,102]. Wyn Jones [74] suggested that its distribution in plants has taxonomic relevance [78, 103,104].

Glycine betaine is found in high amounts in salt-tolerant species, and in low amounts in salt-sensitive plants [92]. However, its levels showed considerable increase in salt sensitive spinach leaves under salt stress [74,105]. Localization of glycine betaine in cytoplasm was demonstrated by subcellular fractionation [106] and histochemical studies [107].

Accumulation of glycine betaine occurs predominantly under gradual stress, whereas proline accumulated under highly damaging and lethal salt levels [94, 101,108]. Unlike proline, glycine betaine is not degraded rapidly [74,92,109].

Other betaines, for example β-alanine betaine (homobetaine) [110,111], dimethyl propiothetin [112], and proline betaine [113] have been reported to function as osmoregulators. It has been shown that levels of asparagine [89] and ureids (allantoin and allantoic acid) [114] increased under salt stress. Sugars (in Juncaceae and Cyperaceae) [103,115] (in halophytes) [116], and sorbitol (in *Plantago maritima*) [117], and (in *P. coronopus*) [118], also play the role of osmoregulators under salt

stress. Recently, diamines like putrescine were shown to increase in their amounts in leaf cells and protoplasts under osmotic stress (0.4 to 0.6m sorbitol) [119] or salt stress [120].

SALT TOLERANCE THROUGH BREEDING

Traditionally, the development of new crops has depended on the efforts of the plant breeder. The identification and isolation of salt-tolerant germplasm in related species of existing crop plants is a basic requirement in a breeding program for salt tolerance. Genetic differences for salt tolerance have been reported in tomato, wheat, barley, *Agropyron*, and rice [60, 121-129]. Breeding for salt tolerance was emphasized by Epstein and Jeffries [130], Norlyn [131], Ramage [132], and Epstein et al. [122]. Subsequently, information was collected and compiled by the USDA [133], which listed over 1500 species that exhibited some degree of salt tolerance (see also Croughan and Rains [134]). However, the breeding programs conducted so far have been inadequate. While reports by Akbar et al. [135], and Akbar and Yabuno [136,137] clearly indicated inheritence of salt tolerance in rice, most existing tolerant crops have come accidentally into agricultural use as a result of breeding experiments for other characters [132].

Success in breeding for characteristics such as salt tolerance depends on selecting the background genotype that is most favorable for the expression of a gene or combination of genes. Retention and expression of other gene clusters, such as those involved in biomass inheritance, also need to be considered; there is no profitable advantage in selecting salt-tolerant crops which have greatly reduced yields. One of the methods used in conventional breeding plans (e.g., corn) is that of recurrent selection [132]. This selection technique can be used as well for salt tolerance selection (Figure 1).

The major draw-backs of a breeding program are: 1) the time required for effective selection, and 2) the difficulty in maintaining the background genotypes for maximum gene expression. Recurrent selection also is difficult to apply in self-pollinating crops.

To date, very few salt-tolerant crops have been obtained from conventional breeding methods. As an example, when tomato variety *Lycopersicon cheesmanii*, a salt-tolerant species of wild tomato [121,138], is crossed with commercial tomato, some backcross progeny can grow in 70% sea water and produce highly flavorful cherry-sized fruits [122].

SCREENING OF PLANTS FOR TOLERANCE

Wild populations of existing crops can be screened for tolerance to salt, and resistant forms can be selected for further studies. Progeny obtained from breeding or

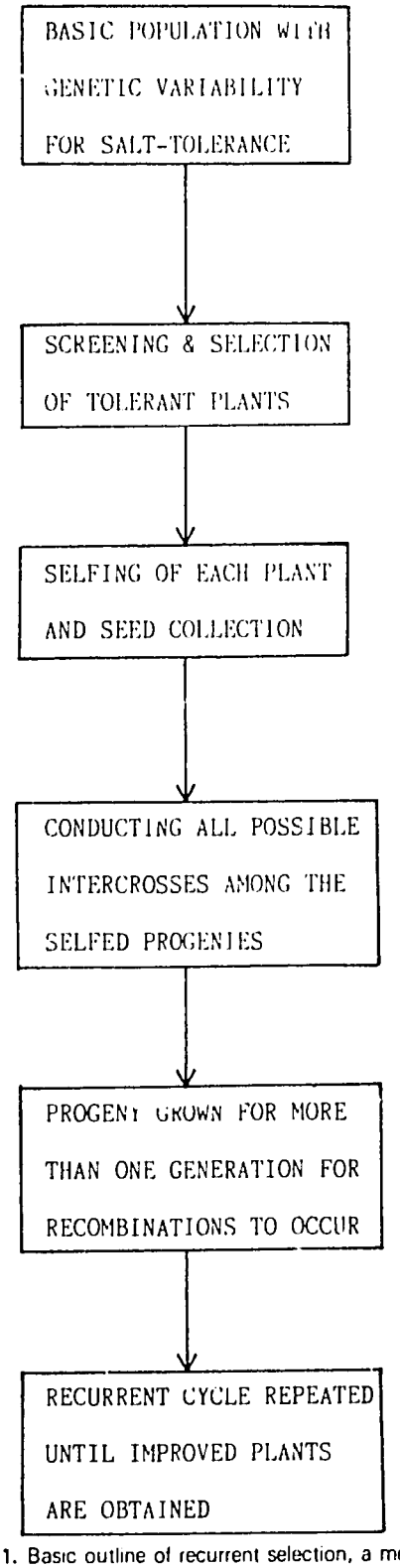

FIGURE 1. Basic outline of recurrent selection, a method for obtaining stress-tolerant plants through traditional breeding methods.

tissue culture selection systems need to be tested for their level of tolerance. Screening of drought- and cold-tolerant plants is also important since drought- and cold-tolerant plants also presumably exhibit salt tolerance [132].

Screening for tolerance can be done by evaluating the effects of salinity at different stages of growth and development, for example, germination, emergence, seedling, tillering, and mature plant stages. However, it appears that most authors prefer screening at the seed germination stage, as that is the first step of a plant's life on saline soils. If a seed cannot germinate under saline conditions, subsequent plant tolerance is of no value in agriculture. However, tolerance at any one stage of plant life does not necessarily indicate its tolerance throughout the life cycle [131,139,140]. Seedling growth assays are also commonly used [141].

Salinity response can be screened in plants germinated and grown in many different soils, in containers or in the field [142,143]. Different methods for screening are described by Nieman and Shannon [144]. Sea water can be used in screening germinating seeds as well as in irrigation of experimental fields; however, prepared salt solutions (NaCl) are more convenient and precise.

Norlyn [131] screened seeds of different varieties by germinating them on a nutrient medium containing 400mM NaCl in 700 liter tanks with plastic grates. Seeds from his selected plants were subjected to a secondary screening involving completion of a life cycle under salt stress. At IRRI, pre-germinated seedlings were transplanted to a soil slurry containing salt [129,145]. Responses were recorded as the number of dead leaves versus the total number of leaves for each plant. Moeljopawiro and Ikehashi [129] demonstrated the possibility of using this technique for selecting progeny lines more tolerant than the parents.

More recently, Jones and Stenhouse [146] developed a method to rapidly evaluate rice varieties and breeding lines for salt tolerance, by comparing seedling root growth in salinated and non-salinated culture solutions. Root lengths of seedlings were measured seven and eleven days after germination. The seedlings were then placed in saline (80mM NaCl) culture solutions. Root length was measured 13 and 17 days after germination. Salinity tolerance was calculated as the tolerance ratio (TR), i.e., root growth after four days in saline solution to root growth after four days in non-saline solution. From this study they described a range of -0.46 (roots of some very sensitive cultivars shrunk in the saline solution) to +0.52. One variety (Pokkali) recorded a tolerance ratio of 0.52 as most tolerant. Eleven varieties had tolerance ratios larger than 0.40, 46 varieties were moderately tolerant (TR > 0.15), and 453 varieties exhibited low tolerance (TR < 0.15) (of these, 342 showed lower than 0.04).

In our lab, we have adopted the nutrient flow technique (NFT) for screening seedlings and regenerated plants from tissue culture [147]. NFT is basically a hydroponic system in which plants are grown in narrow trough down which a controlled nutrient solution flows continuously [148]. The important point of any stress tolerance testing system is that plants can be evaluated for resistance throughout their life cycle and over a range of stress levels. For many plants, aeroponic systems (vividly demonstrated at Disney World's EPCOT Center, Orlando, Florida) are also worthy of consideration. It is important to include a standard indicator cultivar in each test so that results from different experiments can be standardized.

TISSUE CULTURE SCREENING AND SELECTION

Aside from conventional breeding and mutation induction using seeds or other plant parts, cultivars with increased salt tolerance can potentially be isolated by several methods.

A. Cell cultures on solid or in liquid medium can be used as a population base from which spontaneous or induced variants with increased tolerance can be isolated by specific selection procedures (Figure 2).
B. Cell cultures can be used as a population base from which somaclonal variants, i.e., genetically different somatic cells obtained without selection, with in-

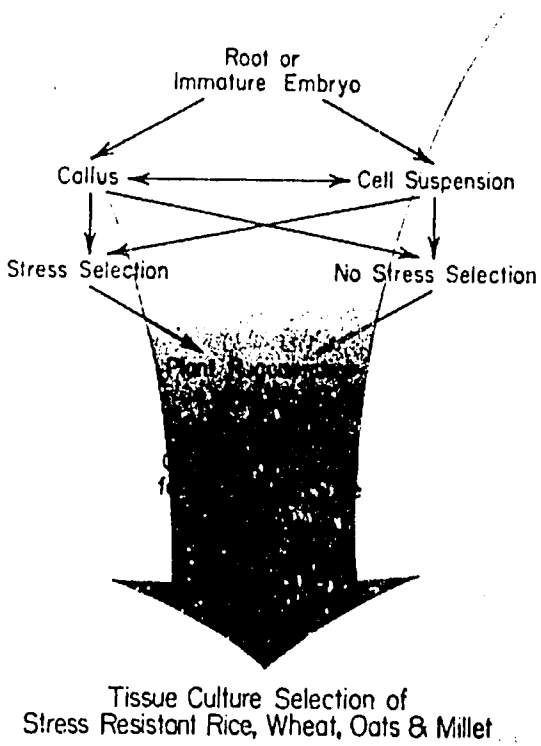

FIGURE 2. A schematic diagram showing how tissue culture and selection is utilized to produce stress tolerant plant cultivars.

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creased tolerance can be obtained without specific selection procedures.

C. Populations of protoplasts obtained from whole plants or cell cultures can be used as a source of somaclonal variants with increased tolerance.

D. Techniques of gene isolation and transfer can be used to directly move genes for increased tolerance from one cultivar, species, or genus, etc., to another.

The first three methods are now within the range of possibility or have already been accomplished for some plants. Gene isolation and transfer is at present beyond the range of possibility for reasons which will be briefly mentioned.

A. Genes regulating salt tolerance have not been identified in higher plants let alone mapped or sequenced.

B. Techniques for isolating, identifying, transferring, and regulating the expression of higher plant genes are at present primitive, although quite rapid progress is occurring.

Production of variants with increased salt tolerance has not been reported from isolated protoplasts, although many other types of somaclonal variants have been produced in a few species [149,150]. The source and type of alterations in somaclonal variation is a subject of current debate. In terms of protoplasts produced from leaf mesophyll cells, the variants could be inherent in the cells themselves, perhaps caused by natural radiation, somatic recombination, mis-repair of DNA damage, etc., or could be induced by the protoplast isolation, culture, and regeneration procedures. Some variants have been shown to be Mendelian in terms of inheritance; whether point mutations or chromosmal mutations (rearrangements) are involved is not known. Protoplasts isolated from mature plants, as well as tissue culture techniques and media, increase stress on plant cells and expose them to potentially mutagenic chemicals.

The current literature (as of October 1983) on salt-tolerant cell lines and regenerated plants is summarized in Table 2. The usual method for obtaining salt-tolerant cell lines has been direct selection, i.e., adding NaCl to liquid or solid medium to obtain a differential growth and division rate between tolerant and non-tolerant cells in the population.

Mutation-inducing chemicals have been used by some workers, and while they increase the frequency of salt-tolerant variants, are not required to obtain variants if large cell populations are used. Our data from tobacco suspension cultures suggest that salt-tolerant variants occur spontaneously in one of every 10^3 to 10^4 cells. R. A. Simons, University of Arizona, has produced a mathematical model (publication in preparation) for mutant selection in plant cell suspensions which can be used to estimate mutation rates from growth data.

The fact that small (10^6 cells) populations of normal somatic ploidy for several species have been used to obtain NaCl variants is strong circumstantial evidence that any of these tolerant variants which are characterized as Mendelian mutants will be dominant or co-dominant alleles. Anther-derived cultures are useful to obtain recessive alleles provided the species are not of polyploid origin (e.g., tobacco and wheat). In the case of allopolyploids, anther-derived polyhaploid cells may or may not have one copy of a particular allele. For example, Carlson [201] found that his auxotrophic lines derived from haploid tobacco were leaky, indicating the possible presence of other non-auxotrophic alleles.

Three critical demonstrations are of interest for variant cell lines produced from tissue cultures: first, the tolerance must be phenotypic in whole plants as well as cultured cells; second, the tolerance must be inheritable in whole plants and the pattern of inheritance must be demonstrated; third, it must be shown that the selected tolerance is stable in the absence of stress.

With reference to Table 2, the following categories of salt tolerance can be noted:

STABLE CULTURES

Barnett [159]
Ben-Hayyim and Kochba [152,153]
Bhaskaran et al. [155]
Conrad et al. [158]
Dix and Pearce [162]
Dix and Street [163]
Kochba et al. [173,174]
Nabors [147]
Nabors et al. [179,180]
Rangan and Vasil [184]
Sobko et al. [188]
Tyagi et al. [191]
Warren and Gould [195]
Yasuda et al. [199]

TOLERANT CULTURES--STABILITY NOT TESTED

(not grown in absence of salt, then recultured on saline media; but can grow for lengthy period on saline media.)

Barlass and Skene [151]
Chen et al. [157]
Croughan et al. [160,161]
Handa et al. [167]
Heyser and Nabors [170,171]
Ju and Rains [172]
Komizerko and Khretenova [175]
Liu and Yeh [177]
Nyman et al. [181]
Orton [182]
Oshmarina and Shamina [183]
Strogonov [189]
Tal et al. [190]
Wenzhong et al. [196]
Zenk [200]

UNSTABLE CULTURES

Bressan et al. [156]
Gale and Boll [164]
Hanson [168]
Hasegawa et al. [169]
Kurtz [176]
Smith and McComb [186]
von Hedenström and Breckle [193]
(cellular test for halophytes)

REGENERATED PLANTS—NO DATA ON STABILITY OR TOLERANCE

Barnett [159]
Bhaskaran et al. [155]
Conrad et al. [158]
Handa et al. [167]
Nyman et al. [181]
Tyagi et al. [191]
(callus from regenerate tolerant)
Wong et al. [197]
Yasuda et al. [199]

TABLE 2. Flowering Plants in Which the Tissue Culture Techniques Have Been Used in Relation to Salinity.

Reference Author(s)	Plant(s)	Initial Tissue	Selection Tissue	Salt Concentration	Selection Time	Stability of Tolerance Determination
Barlass and Skene, 1981 [151]	*Vitis* sp. cultivars	shoot tip (liquid and semi-solid media)	apical fragments and derived proliferating shoot cultures	0,5,20,50,100, 200mM NaCl	9 months	Varietal difference in tolerance to NaCl is detected.
Ben-Hayyim and Kochba, 1982a [152]	*Citrus sinensis* (Shamouti)	ovular callus	callus and suspension	0.2M NaCl (Mutagen = gamma radiation)	10 passages (30 days/ passage)	*Stable*. Cells maintained in no-salt for 4 passages still retained tolerance.
Ben-Hayyim and Kochba, 1982b [153]	*Citrus sinensis* (Shamouti)	ovular callus	callus	0.2M NaCl	selected cells maintained for over 1 year	*Stable*. Tolerant cells were more sensitive to absence of Ca^{2+} and presence of KCl in the medium than the non-selected cells.
Ben-Hayyim and Kochba, 1983 [154]	*Citrus sinensis* (Shamouti)	ovular callus	callus and suspension	0.2M NaCl (Mutagen = gamma radiation)	10 passages	*Stable*. Retained tolerance after 4 passages on no-salt media. Correlated also from data on ion uptake.
Bhaskaran et al., 1983 [155]	*Sorghum bicolor*	seedlings	callus	0-86mM	10 months	*Stable*. Plants regenerated from 8 month old callus in stress medium. 10 month old callus continued to grow in medium with NaCl
Bressan et al., 1981 [156]	*Lycopersicon esculentum* Mill. cv. VENT cherry	callus	suspension	15% PEG	14-50 days	*Unstable*. Cells selected for PEG tolerance also exhibited salt tolerance. Lost their tolerance when transferred to medium without stress (PEG).
Chen et al., 1980 [157]	*Nicotiana tabacum*	callus line 44	callus	0-4.4% seawater powder	3-4 weeks	Primarily a study on short-term growth kinetics. Stability not determined.
Conrad et al., 1983 and Barnett, 1983 [158,159]	*Lycopersicon peruvianum*	not stated	callus	50% seawater (mutagen = EMS)	3 months	*Stable*. Cells cultured on a medium without seawater, for 1 month, retained tolerance after transfer to seawater medium. Plants were regenerated from resistant callus.
Croughan et al., 1978 [160]	*Medicago sativa* var. W74-RS	cotyledonary callus and suspension	suspensions planted on agar medium	1% NaCl	8 months	*Stable*. Selected cell lines exhibited tolerance when maintained on 1% NaCl medium for another 6 months.
Croughan et al., 1981 [161]	*Oryza sativa* var SD7 (haploid and diploid)	haploid and diploid vegetative portions	callus	0,0.5,1.0,1.5% NaCl	not stated	Selected calli were tolerant up to 1.5% NaCl. These required 0.5% NaCl for optimum growth.

(continued)

TABLE 2. (continued).

Reference Author(s)	Plant(s)	Initial Tissue	Selection Tissue	Salt Concentration	Selection Time	Stability of Tolerance Determination
Dix and Pearce, 1981 [162]	*Nicotiana sylvestris*	haploid plant	suspensions	0 and 1.5% NaCl	used selected cell lines from Dix and Street (1975)	Studies on proline accumulation under stress. No test for stability. Both cell lines (selected and non-selected) possessed proline
Dix and Street, 1975 [163]	*Nicotiana sylvestris* and *Capsicum annuum*	suspensions	plated on agar and suspensions	0.7, 1.0, and 3.0% NaCl	6 weeks in Petri dishes, 21 days in suspension	*Stable*. Retained tolerance to 1% NaCl after 3 passages on no-salt medium. Suspensions maintained for 10 passages exhibited improved growth comparable to controls
Gale and Boll, 1979 [164]	*Phaseolus vulgaris* cv. Contender	cotyledonary callus	suspensions	0 to -4 bars NaCl (gradually increasing salt concentrations)	0 to -4 bars for 9 days, then 6 passages (8-13 days each) on 0 or -4 bars NaCl	*No tolerance*. No increase in tolerance to NaCl after 6 transfers through salt.
Garcia and Einset, 1983 [165]	*Nicotiana tabacum* cv. Wisconsin 38		callus	0 and 200 $Meq l^{-1}$ NaCl		Not a selection study.
Goldner et al., 1977 [166]	*Daucus carota* var. Chantenay, var. W93-A	hypocotyl	callus	0-60% seawater; synthetic seawater	3 passages (30 days/passage)	Primarily physiological studies; stability not determined. (Selected cell lines were considered as survivors.)
Handa et al., 1982 [167]	Tomato		suspensions	3.5% NaCl	not stated	*Stable*. Tolerance differences were detected between tolerant and non-selected cell lines.
Hanson, 1983 [168]	*Lycopersicon esculentum* and *L. pennellii*	hypocotyl or stem sections	callus	0.8 and 1.2% NaCl	not stated	No tolerance in cultures of tolerant variety.
Hasegawa et al., 1980 [169]	*Nicotiana tabacum* var. Wisconsin 38	stem callus	suspensions	0-25g/l NaCl (10g/l NaCl selection level)	40 cell doublings	*Unstable*. After 5 cell doublings in no-salt media selected cells lost tolerance to salt in media.
Heyser and Nabors, 1981a,b [170,171]	*Nicotiana tabacum* var. Samsun	internode segments	suspension	0-17mM NaCl	approximately 48 weeks selection and then maintained for 10 months at 130mM NaCl	Studied ion accumulation and osmotic adjustments in adopted and non-adopted cell lines.
Ju and Rains 1983 [172]	*Oryza sativa* var. SD7	roots	suspensions plated on agar medium	1.5% NaCl	11 months	Stability not reported. Albino plants were regenerated.

(continued)

TABLE 2. (continued).

Reference Author(s)	Plant(s)	Initial Tissue	Selection Tissue	Salt Concentration	Selection Time	Stability of Tolerance Determination
Kochba et al., 1980 [173]	*Citrus sinensis* (Shamouti)	ovule	callus	5gm/l NaCl	10 passages (5 weeks each)	*Stable*. Callus cultured in the absence of NaCl for 1-3 passages retained tolerance on return to 5g/l. (Tolerance is also retained by cells for over 6 months.) Embryoids were differentiated.
Kochba et al., 1982 [174]	*Citrus sinensis* *C. aurantium*	ovular callus	callus and suspension	5g/l and 7g/l (Mutagen= gamma radiation)	10 passages of 5 weeks each at 5%, 10 passages of 3 weeks at 7%	*Stable*. Cells after 3 passages without salt grew on 5-10g/l. Embryoids were differentiated
Komizerko and Khretonova, 1973 [175]	*Daucus* sp.	root	callus	(0,1.4,3.7,5.7 atm)(0,0.2,0.5, 0.7% NaCl)	4 passages (35-45 days/ passage)	Plants regenerated from cambial callus are more tolerant than those derived from xylem or phloem callus. Both salt-selected and control plants derived from cambial callus showed tolerance to 4.4 atm.
Kurtz, 1982 [176]	*Lycopersicon esculentum*	leaf explants and cotyledons	callus	0,0.5,1.0,1.5,2.0, 2.5,3.0% NaCl	5 weeks	Studied growth data and NaCl effect. No tolerant cell lines were isolated.
Lui and Yeh, 1982 [177]	*Saccharum* sp. cvs. F108 and F156	callus	suspensions and callus	0 to 1.5% NaCl (stepwise salinization increments of 0.12% up to 1.5%)	4-8 passages (7 days/passage)	Cells expressed tolerance. No regeneration. No test for stability.
Mathur et al., 1980 [178]	*Kickxia ramosissima*	internode segment and leaves	internode segment and leaves	20,120, and 240mM NaCl; >120mM NaCl inhibited most cultures	first passage bud initiation, then subsequent shoot formation on salt medium – maintained for 20 months on 120mM NaCl	*Stable*. Plantlets transferred to no-salt medium for 3 passages (8-10 weeks each) – showed tolerance to 120mM under stress.
Nabors, 1983 [147]	*Oryza sativa* *Triticum* *Avena sativa* *Pennisetum*	seed	callus	0.05, 0.1, 0.15mM	>12 months	*Stable*. Oat plants regenerated from callus showed tolerance in F_1 and F_3 generations.

(continued)

TABLE 2. (continued).

Reference Author(s)	Plant(s)	Initial Tissue	Selection Tissue	Salt Concentration	Selection Time	Stability of Tolerance Determination
Nabors et al., 1975 [179]	*Nicotiana tabacum* var. Samsun	callus from stem sections	suspensions	1.6g/l NaCl	11 weeks	*Stable*. Increased tolerance in cells up to 5.2 g/l
Nabors et al., 1980 [180]	*Nicotiana tabacum* var. Samsun	callus	suspension	As reported in Nabors et al., 1975		*Stable*. Increased tolerance up to 8.8g/l. Regenerated plants F_1 and F_2 generations exhibited stable tolerance. Tolerance was increased in whole plants (to 33.4g/l NaCl)
Nyman et al., 1983 [181]	*Colocasia esculenta* var. *antiquorum* (L.) Schott	shoot tips	shoot tips in liquid or on semi-solid media produced callus	0,10,20,30,40,50, 60,70% ASW (gradual-10% increment every 2.5 months)	32 weeks	*No study for stability*. Plantlets developed in all concentrations. Optimum for regeneration - 40-50% (ASW). Plantlets died after 6 months in soil
Orton, 1980 [182]	*Hordeum vulgare* *H. jubatum* Hv × Hj hybrid	immature ovary wall callus	callus	0.0,0.017,0.17, and 0.86M NaCl	30 days on 0-salt, then 30 days on salt, then 30 days on 0-salt	Tolerance of whole plants and callus of *H.j* was more than that of *H.v*—hybrids formed intermediate in their tolerance.
Oshmarina and Shamina, 1982 [183]	*Nicotiana sylvestris* (haploid)	mesophyll cell cultures derived from several plant parts	suspensions	0.1-2% NaCl	not stated	Selected lines grew at 1% NaCl. No stability test was mentioned.
Rangan and Vasil, 1983 [184]	*Pennisetum americanum*	inflorescence callus	suspension	10^{-3} to 2×10^{-1}M (.0058 to 1.16%)	12 months (50 passages) (gradual increase in salt levels)	*Stable*. Cells transferred to medium without NaCl for 7 passages (2 months) retained tolerance.
Rosen and Tal, 1981 [185]	*Lycopersicon esculentum* *L. peruvianum*	leaves	protoplasts	0,0.1,0.15,0.2, 0.25,0.3,0.35% NaCl (gradual increase in salt)	2-3 weeks	Plating efficiency on NaCl-containing medium is greater in *L. p.* than in *L. e.* No regeneration. No selection for tolerant protoplasts. Tolerance exhibited by whole plants is reflected in the plating efficiency of their protoplasts.
Smith and McComb, 1981 [186]	*Phaseolus vulgaris, Beta vulgaris, Atriplex undulata, Suaeda australis*	hypocotyls	callus	0.1-200mM NaCl (gradual increments not exceeding 50mM)	28 days	Halophytes *A. u.* and *S. a.* did not exhibit tolerance in callus cultures. Tolerant glycophites exhibited some tolerance in callus cultures. *No regeneration*—not a selection study.

(continued)

TABLE 2. (continued).

Reference Author(s)	Plant(s)	Initial Tissue	Selection Tissue	Salt Concentration	Selection Time	Stability of Tolerance Determination
Smith and McComb, 1983 [187]	*Medicago sativa*	suspensions	plated on agar	0 1,62 5,125, and 250mM NaCl	2 months – screening repeated 5 times	*Unstable*. Plants regenerated from cell lines on 62.5mM NaCl did not show any tolerance.
Sobko et al., 1981 [188]	*Crepis capillaris*	stem derived cell culture	callus	0,0.05,0.1,0 2, 0 4,0.6,0.8,1.0% NaCl (gradually increasing concentrations)	5-6 passages at each NaCl level (4 weeks/ passage)	*Stable*. Maintained on NaCl medium for 13-15 passages – cells adapted to 0.7% NaCl. Callus on medium without salt for 4 passages retained tolerance.
Strogonov, 1973 [189]	a) alfalfa	root cultures 27 passages old (of 1 week each),	root cultures	0.1-1.1% NaCl (gradual) increments of 0.1% at each passage),	80 days	No increased tolerance in roots.
	b) carrot	isolated tissues from phloem and xylen parenchyma and cambium,	callus	0-6 9 atm NaCl	30 days	Callus of cambial origin was most tolerant among the different sources.
	c) cabbage, tobacco, sweet-clover, sorghum and glasswort	different plant parts	callus	0,0 35,0.7,1.4, 2.2,2.9,4.4,5 7, 7.0,8.6,9.3 atm NaCl or $NaSO_4$	not stated	Salt tolerance of callus tissues from different plants was unrelated to the natural salt tolerance.
Tal et al., 1978 [190]	*Lycopersicon esculentum* cv. Rheinlands Ruhm. *L. peruvianum*, *Solanum pennellii*	leaves, stems, roots	callus	0.0,0 5,1 0,1.5, 2.0% NaCl	1 month	Tolerance in whole plants was comparable to tolerance expressed in culture.
Tyagi et al., 1981 [191]	*Datura innoxia* Mill. (haploid)	stem explant	callus	1% for 3 subcultures to select tolerant cell line, then 0-2% for 1 month	3 months	*Stable*. Retained tolerance when cultured without NaCl for 1 month. Callus from regenerated plants possessed tolerance after 5 months without salt.
Umiel et al., 1980 [192]	*Nicotiana* sp.	callus	callus	0.0,0.4,0.8,1.2, 1.6,2.4,2.8% NaCl	0.5-48 hours	Short term kinetic studies on ion uptake.

(continued)

TABLE 2. (continued).

Reference Author(s)	Plant(s)	Initial Tissue	Selection Tissue	Salt Concentration	Selection Time	Stability of Tolerance Determination
von Hedenström and Breckle, 1974 [193]	*Suaeda maritima*, *Salicornia europea*	seedlings	callus	0.5,0.75,1.0,3.0, 5.0% NaCl	10 days	Use of callus cultures for study of salt sensitivity among halophytes is recommended
Wardle et al., 1981 [194]	*Brassica oleracea* L. var. *botrytis* cv. Barrier Reef		seed, seedlings and regenerated plants	0-5gm/dm³	28 days	Studied ion accumulation. K:Na ratio decreased in the order of seedling > seed culture > regenerated plants at any given NaCl level.
Warren and Gould, 1982 [195]	*Distichlis spicata*	immature rachis and flowering head	suspension	20,43,85,170,260, 340,430,550,685, 850mM	9 months	*Stable*. Growth unaffected up to 170mM. Tolerance stable without selection pressure for 3 years.
Wenzhong et al., 1981 [196]	*Oryza sativa* sub sp. Keng	anthers and pollen	callus	0.3-3% NaCl (Mutagen=EMS)	30 day minimum	*Stable*. Plants regenerated on 0.5, 0.8% grew on 1%, showing increased tolerance. Seeds were obtained. No further tests were made.
Wong et al., 1983 [197]	*Oryza sativa* cv. Tainan 5	anthers	haploid callus	0.6,1.0%	not stated	Not mentioned if any regenerated plants possessed tolerance.
Yano et al., 1982 [198]	*Oryza sativa* cv IR36	mature embryos	callus	17.5,27.5,37.5, 47.5,57.7,67.5% seawater (SW)	16 weeks	*Unstable*. Plants regenerated from calli selected on 37.5% SW. Plants regenerated from calli selected on 17.5% SW grew on 17.5% SW. Second generation plants lost characteristics of tolerance with regard to Na and K ratio.
Yasuda et al., 1982 [199]	Sugarcane NCO310, *Oryza sativa* cv. Chiem chana, *Coffea arabica*	shoot segments and leaves	callus	1.5% NaCl	40 days	*Stable*. Tolerance increased to 2.0%. Sugarcane regenerated on salt medium. Coffee did not regenerate. No mention of tolerance in plants after transfer to soil
Zenk, 1974 [200]	*Nicotiana sylvestris* (haploid)	suspensions	suspensions	1% NaCl (gradually increasing concentrations)	15 passages (24 days/passage)	*Stable*. Cells grew at 1% NaCl for 15 passages, controls did not grow at 1% NaCl

REGENERATED PLANTS STABLE

Goldner, et al. [166] (tolerance tested for 90 days)	Mathur et al. [178]
	Nabors [147]
	Nabors et al. [180]
Komizerko and Khretenova [175]	Wenzhong et al. [196]

REGENERATED PLANTS UNSTABLE

Smith and McComb [187]	Yano et al. [198]

TOLERANCE IN REGENERATES INHERITABLE

Nabors [147]	Nabors et al. [180]

Salt-tolerant plants with demonstrated stable, inheritable tolerance have been regenerated from tolerant cultures by our group for tobacco [147,180] and oats (Figure 3) [147]. A consistent problem in the literature is that some workers do not report the length of selection and do not carry out experiments to determine if tolerance is stable. Considerable variation exists in methods for testing the tolerance of whole plants. In some cases, tolerance is reported without a discussion of methods utilized. In addition, the length of exposure to non-salt-containing medium necessary to demonstrate stable tolerance has not been determined.

In general, the issue of the origin of tolerance stability in both regenerated plants and cultured cells is unresolved (see Table 2). In our tobacco cell lines and in the literature, tolerance seems to be related to the length of selection, with periods of six to twelve months selection (in medium allowing almost no growth of non-tolerant cells) required before stable tolerance is achieved.

Handa et al. [202] conducted extensive experiments on cell suspensions of tobacco tolerant polyethylene glycol 4000, an osmotically active molecule which competes with cells for available water. They concluded that resistant cultures were physiologically, but not genetically, adapted and that selection of tolerant genotypes had not occurred. Tolerance was not stable in the absence of stress. Their group has obtained similar results for NaCl-tolerant cell suspension (reported at 1983 Annual Meeting of the American Society of Plant Physiologists, Ft. Collins, Colorado). The data support the conclusion that physiological adaptation of tolerant cells to stress may occur rapidly enough so that tolerant mutant cells do not have a selective advantage.

Curiously, when small clumps—presumably individual clones—were cultured separately, considerable differences in tolerance were found, indicating that through somaclonal variation, the suspension does indeed contain variation in tolerance. Whether this variation is genetically based could not be rigorously determined from cell studies alone. However, the Luria-Belbruck Fluctuation test (which allows the separation of physiological adaptation from genetic differences in tolerance) indicated the variation was due to mutation.

It is tempting to hypothesize that selection did not occur in the experiments of Handa et al. [202] because the length of exposure to stress was not long enough nor the level of stress high enough to give genetically adapted cells a selective advantage over physiologically adapted cells. Whether or not this is indeed the case can only be determined by future experiments to measure the upper

FIGURE 3. F_3 oats derived from parental plants obtained from callus cultures. From left to right plants were watered with solutions containing 0.0, 5.0, and 10.0 g/l NaCl respectively. Front row is plants derived from cell lines not exposed to NaCl. Back row is plants derived from cell lines selected for tolerance to 3 g/l NaCl.

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limits of non-genetic physiological adaptation versus that for genetic tolerance. In general, the fact that the tolerance of halophytes is genetically based would seem to argue that this is a more efficient, effective mechanism of tolerance than simple physiological adaptation without genetic change. At low levels of selection pressure, it is likely that physiologically adapted cells could compete favorably with those having a genetically increased tolerance. At higher levels of selection, however, and particularly for long-term selection, genetically tolerant cells would seem to have the selective advantage. If this analysis is correct, it argues strongly for selective pressure which drastically restricts the growth and division of most cells and which is gradually, or in a stepwise fashion, increased over time. Long-term selection is important to obtain homogeneous mutant cultures and to eliminate the possibility (of debated relevance [147]) of obtaining chimerical regenerated plants.

In our selection experiments with tobacco suspensions, nine increasing levels of NaCl were used in successive selections before plants were regenerated of cell lines tested for stability [147,180]. The plants are tolerant and both cell lines and plants retain tolerance in the absence of stress.

In no case has the genetic mechanism of inheritance or physiological base of selected salt tolerance been rigorously determined. At present, the lack of this information constitutes a most serious gap in basic studies which will ultimately impede the practical utilization of salt-tolerant variants in plant breeding. In the case of our NaCl-tolerant tobacco plants, available data do not support the hypothesis that a single Mendelian allele is involved [180]. This conclusion is not surprising in view of the lengthy, step-wise selection employed to obtain the variants. Information to clarify the situation from backcrosses has, unfortunately, not yet been obtained.

An important issue in tolerance selection at the cellular level is the fidelity of correspondence between cellular and whole plant tolerance for the same species. Some data seem to show clearly that a close match occurs in the two tolerances while other data show no correlation.

In the first group are the data of Warren and Gould [195] who showed that cells of *Distichlis spicata* (a monocotyledonous halophyte) in suspension cultures tolerated salt levels at least as high (if not higher) than whole plants in soil [203]. Also, Tal et al. [190] compared the callus growth of a cultivated, salt-sensitive tomato and two wild, salt-tolerant relatives and found that callus of the tolerant cultivars was much more resistant. In addition, they [185] reported a similar correspondence in protoplast plating efficiency on media containing NaCl. Orton [182] arrived at similar conclusions for a tolerant and sensitive species of barley.

Some data indicate a partial correlation between tolerance of calli and entire plants. Thus, citing Flowers [7] and von Hedenström and Breckle [193], Warren and Gould [195] point out that *Suaeda maritima* and *S. europea* cells in culture grew at optimal levels of about 85mM and 170mM, respectively, which were in general less than, but positively correlated with the optimal levels for whole plants in soil. At concentrations of salts corresponding to optimal soil, cell growth was drastically affected in suspended calli [193]. This, they conclude, may be due to contributions of succulence and salt glands and other anatomical and physiological specializations in addition to the cellular basis of tolerance. It is important to note that this conclusion is obtained by comparing data from one paper about callus growth alone and several (summarized in Flowers et al. [7]) about whole plant growth alone.

A second group of data show no correspondence between salt tolerance of calli and whole plants of the same species. For example, Smith and McComb [186] compared the salt tolerance of a salt-sensitive glycophyte, a tolerant glycophyte, a sensitive halophyte, and a tolerant halophyte. They found that growth of whole plants in increasing concentrations of salts decreased in the expected order from the salt-sensitive glycophyte, which was most affected, to the salt-tolerant halophyte, which was least affected. In the glycophytes, callus growth (in terms of percent fresh weight) in salt-containing media was exactly the same as whole plant growth. In the halophytes, however, callus was considerably more sensitive to salt than whole plants and equalled growth of the glycophytic callus. Also, Strogonov [189] failed to observe any differences in the salt tolerance of callus cultures of a halophyte and several glycophytes.

Thus, the tissue culture data agrees with physiological studies and suggest the existence of at least two general mechanisms for dealing with excess salt: a cellular mechanism which results in correspondence between tolerance of calli and whole plants, and an organismal mechanism which does not result in correspondence.

The relative growth rates of calli in basal media (containing no NaCl) is also important in comparing species. Hanson [168] compared the salt tolerance of callus, roots, and shoots of a salt-sensitive, salt-tolerant, and a hybrid tomato cultivar. She found that *in vitro* tolerance could not be correlated with reported whole plant tolerances in terms of percent fresh weight increase. In terms of actual fresh weight, the correlation could be made, but the calli which were most salt-tolerant were also those which grew most rapidly in medium containing no salt. Tal et al.'s [190] figures show similar data in some cases. These results suggest simply that "when you're hot, you're hot." Hanson [168] correctly believes that some apparent selections for salt-tolerant tissue cultures may only be selections for increased growth rates on non-salt-containing media. On the other hand, selections for vigorous growth might also give rise (less reliably, apparently) to salt-tolerance. We have observed that salt-tolerant tobacco calli (from cell selections) grow more than twice as rapidly than salt-sensitive calli in basal medium [147]. No growth differences were observed between the two groups of whole plants when watering

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solutions contained no NaCl; however, differences did appear as salt concentrations increased [180].

A final consideration when comparing the salt tolerance of whole plants and cultured cells concerns the method of cell culture. For Samsun tobacco, we have found that cell suspensions, calli, and whole plants are decreasingly susceptible to NaCl toxicity. For example, a suspension which tolerates 9.4 g/l NaCl will produce calli tolerant to 17.2 g/l and whole plants tolerant to 26.2 g/l. Since suspensions and calli are rather anatomically disorganized, these data suggest that accessibility to the salt is an important consideration in determining tolerance. Our relatively more salt-tolerant calli in tobacco gave rise to relatively more salt-tolerant plants, but exact comparisons between salt concentrations in tissue culture and those used to water whole plants were not particularly meaningful.

CONCLUSION

From the literature it is evident that conventional breeding methods have been neither systematically used nor particularly successful in producing salt- (NaCl) tolerant cultivars of crop plants. Such plants are a critical need in drier regions of the world, particularly those irrigated areas which produce large amounts of our food supply. Current yearly worldwide agricultural loss from salinization of irrigation water sources may exceed one trillion dollars. Tissue culture selection seems to offer a potentially useful complementary tool to assist conventional breeding and field testing to obtain salt tolerance.

The successful application of tissue culture selection to obtain salt tolerance is currently hampered by several gaps in our knowledge and technical abilities.

A. The physiological, biochemical, and genetic bases of tolerance or susceptibility in either whole plants or cultured cells are not well understood.
B. Tissue culture methods to obtain true selection for salt tolerance, as opposed to non-genetically based physiological adaptation or selection for increased growth rate under all conditions, are only beginning to be developed.
C. The factors which lead to stable salt tolerance in selected cell populations are not adequately determined.
D. Inadequate data on obtaining tolerant plants from tolerant cultures is available. Also, the genetic mechanisms of selected tolerance in regenerated plants have not been worked out.
E. The origin and base of salt tolerance from somaclonal variants are unknown, and little data exists on the potential of this method to obtain tolerant cell populations and regenerated plants.
F. For many plant species, inadequate long-term, high-

Until recently, for example, this was the case with cereals; it is still the case for most legumes.

Despite these gaps, sufficient evidence exists to justify the statement that tissue culture can be used to obtain salt-tolerant cell populations and regenerated plants. A focus of research on basic questions, such as those listed above, would ultimately most effectively speed the development of salt-tolerant crops in the field. It is equally correct that experiments to obtain tolerant plants need not be postponed until all theoretical problems have been solved.

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