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Evaluation and Utilisation of Plant Genetic Resources for Salt Affected Soils

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Reclamation of salt-affected soils, based on soil amendments/drainage measures, is both energy as well as labour intensive and, hence, highly expensive. In fact, obtaining and maintaining non-saline soil conditions is not economically feasible where either evapo-transpiration demand far exceeds the precipitation or the area is highly prone to flooding by brackish water or soil permeability is low or water-table is high and groundwater is saline. Slow but continuous build-up of salinity in the root zone threatens agriculture in such situations ; successful farming under these unfavourable conditions centres around proper choice of salt-tolerant crop varieties vis-a-vis *appropriate management practices. It is also noteworthy that even gypsum-based proven technology for reclamation of alkali soils in the Indo-Gangetic plains failed to find favour with the farmers until the advent of high-yielding dwarf varieties of rice and wheat made such efforts remunerative. Success of projects aimed at increasing productivity of salt-affected soils, and improving socio-economic conditions of marginal farmers owning them, is thus linked to collection, conservation, assessment and utilisation of salt-tolerant plant genetic resources.*

Plant species show remarkable variation in their inherent adaptation to different kinds of stress environments (Wright, 1976 ; Staples and Toenniessen, 1984; Epstein and Rains, 1987). It is, hence, logical that traditional farming in salt-affected areas centres invariably on locally adapted and salinity tolerant landraces (Rana, 1977, 1986). Yield potential of these hardy cultivars is, however, very low. Combining higher yield potential with superior salt tolerance is thus the key factor in developing low-cost and low-risk technology for crop production in salt-affected soils. Whereas this breeding objective essentially involves collection, evaluation, manipulation and management of genetic variability for tolerance to adverse soil conditions, it also implies continuing enrichment, conservation, build-up and characterisation of hardy plant genetic resources. A pivotal step towards utilisation of germplasm collections lies, however, in their critical evaluation and this, in turn, requires effective screening procedures and reliable criteria.

CHARACTERISATION OF THE TARGET ENVIRONMENT AND TESTING SITE

Soil-water-plant-atmosphere is a continuum. It is, therefore, necessary for the breeder to monitor important variables and to characterise the target stress environment relevant to his breeding objective(s). He should also collect information regarding the time of onset, intensity and duration of salinity or any other stress factor *vis-a-vis* the crop growth stage. Success of breeding effort will depend to a large extent upon proper matching of plant growth conditions of the experimental set-up with those of the target area and clearly defining the breeding objective(s). It may be pointed out that breeding objectives as well as methodology of germplasm evaluation will be different in case of alkali, saline and acid soils. They will also differ in case of salinity coupled with aridity and salinity associated with water-logging (or inundation by seawater).

The breeder should also bear in mind that it is the sensitivity of the plant that essentially defines the soil problems rather than the assigned values of certain soil characteristics (such as ECe, pH, ESP). A soil having a particular concentration of soluble salts (characterised by an ECe value of 6 or even of 2) may or may not be problematic depending upon the tolerance rating of the plant species (crop varieties) under consideration, soil texture and rainfall among many other factors.

PLANT RESPONSE TO SALINITY STRESS

Salt stress reduces not only the plant's ability to acquire resources (such as water, nutrients and CO_2) but also lowers its ability to utilise these resources (by affecting enzyme activity, cell division, availability of respiratory substrates, etc.).

Osmoregulation, through accumulation of compatible solutes, is heritable cellular adaptation to osmotic stress. In addition, different species and species-groups appear to have developed their own strategies (based on various genetically controlled developmental, anatomical and morphological features) to cope with the demands of saline environment. Ecotypes and cultivars (genotypes) within a species also often show remarkable variations around their specific evolutionary scheme that may substantially extend the range of adaptation of the concerned species.

MEASUREMENT OF SALINITY TOLERANCE

If salt tolerance is defined as the plant's ability to prevent, reduce or overcome the potential injurious effects of soluble salts (toxic ions) present in the root zone, it follows that this advantage should result in a measurable increase in the salinity stress level (salt concentration) required to produce a specified quantum of strain, i.e. a pre-determined level of stress-caused visible injury in the form of tissue death (leaf area damage) or stress-induced reduction in germination/growth/yield relative to non-saline control taken as 100. Salt tolerance is also expected to lead to a measurable decrease in the quantum of strain produced by a specified level of salinity stress.

Testing of salt tolerance in green houses is usually done by awarding visual scores based on per cent leaf area damaged in seedlings grown in media of increasing salinity levels. Salt tolerance of different genotypes is also commonly compared by preparing salinity response curves in respect of key traits (parameters) and then computing value of the conductance of soil saturation extract that corresponds to a specified level of reduction (usually 50%) in seed germination, seedling growth rate, dry matter production, grain yield, etc.

Maas and Hoffman (1977) evaluated published data on salinity-caused yield reductions in 76 crops and prepared their averaged yield response curves. This information was then used for comparing relative salinity tolerance of those crops by computing values for the following two criteria :

(i) Salinity level, beyond which yield begins to decline significantly, is termed the threshold salinity level (SY_{100}), and

(ii) Per cent yield decrement per unit ($dS\ m^{-1}$) increase in salinity beyond the threshold level.

NEED FOR STANDARDISATION OF METHODOLOGY

A survey of literature on the subject reveals that plant response to soil salinity and other edaphic stresses has been studied in the past in many ways and at different growth stages employing several criteria (Strognov, 1962 ; Bernstein, 1975 ; Rana, 1977 ; Maas and Hoffman, 1977 ; Jung, 1978 ; Christiansen and Lewis, 1982 ; Staples and Toenniessen, 1984 ; Pasternak and San Pietro, 1985). A common feature of most of the published reports on this aspect is the lack of standardisation of methods and uniformity of procedures employed in assessment of relative salt tolerance of materials under study. There is an urgent need to prepare a reference manual on relevant methodology and to provide proper guidelines on these basic aspects to workers engaged in salinity researches.

The aforementioned limitations of the published information on relative salt tolerance notwithstanding, there is general agreement on the ranking of different crops in this regard. A part of the inconsistency in computed values of threshold salinity level (SY_{100}) and 50% salinity level causing yield reduction (SY_{50}) in respect of the same crop by different workers could be attributed to lack of control over variables that affect plant response as discussed earlier. In addition, there is often a strikingly large genetic variation regarding salinity tolerance among varieties within a crop.

Plant response to salinity is studied at the CSSRI under partly simulated conditions in lysimeter-type microplots (6m × 3m × 1m) designed for this purpose. Sand culture technique using nutrient solution is also employed. Choice of methodology for assessment of salt tolerance is linked to the study's objective. When comparing salt tolerance of different crops, materials are grown in replication at several levels of soil salinity and also in non-saline (favourable) soil conditions. Yield data are used for computation of SY_{100} and SY_{50} values (Table 1). Donor genotypes for salinity tolerance, within selected crops, are also characterised in this way but additional data are recorded on important component traits that contribute to yield. A different procedure is, however, adopted

TABLE 1. RELATIVE SALT TOLERANCE OF CROPS

Crop Plants	Salinity SY_{100}	(dS m^{-1})SY_{50}
A. *Grain Crops* :		
Mung bean	2.1	4.9
Maize	2.5	7.2
Rice (Transplanted)	5.0	10.3
Pearlmillet	6.0	10.6
Bread wheat	6.2	11.4
Barley	8.1	12.2
B. *Others*		
Sugarcane	4.5	10.0
Sugarbeet	7.7	14.6
Cotton (asiatic)	7.6	14.5
Date palm	5.1	15.0
Berseem clover	2.2	7.5
Alfalfa	3.1	9.4
Paragrass	5.4	10.7
Blue Panic	6.8	12.1
Wheatgrass (Tall)	8.3	18.0

when evaluating germplasm collections of a particular crop or to screen segregating populations, generated through hybridisation, for their salinity tolerance. It is then essential to decide upon a critical stress level that is relevant to the target area situation as well as sufficient to resolve differences in salt tolerance among the genotypes under evaluation for identification of promising materials for more intensive follow-up study. Materials are grown at this selected salinity level and also in non-saline (favourable) soil for comparison of their *per se* yield in saline environment, salinity-caused reduction in yield relative to corresponding controls and also stability of performance. Segregating populations are, however, screened by growing them in saline environment only.

Since genes governing yielding ability (performance *per se*) of a cultivar are expected to be different from those controlling its salt tolerance (survival strategy), the breeder needs to distinguish between these two characteristics. Our experience at the CSSRI shows that inherent advantages with regard to yielding ability under stress environment and the salt tolerance, among materials under study, may be resolved by quantifying these two types of advantages as follows :

Yield Response Advantage (YRA)

$$= \frac{\text{Mean yield of a genotype averaged over all the stress environments}}{\text{Mean yield of all the genotypes averaged over all the stress environments}}$$

Stress Tolerance Advantage (STA)

$$= \frac{\text{Mean yield reduction of a genotype averaged over all the stress environments}}{\text{Mean yield reduction of all the genotypes averaged over all the stress environments}}$$

Computation of YRA values is expected to help the breeder in identification of promising materials for direct cultivation in stress environments. STA values, on the other hand, assist the researcher in spotting of superior genotypes adapted to a specific stress environment and these materials will be of immense value to geneticists and physiologists engaged in understanding heritable differences among related genotypes with regard to specific adaptations.

VARIABILITY FOR SALINITY TOLERANCE

Variation regarding parameters and methodology notwithstanding, published data on response of crop plants to increasing salinity levels show nearly eightfold differences in salt tolerance. Leguminous plants like beans and grasses such as the tall wheatgrass appear at the two extremes of this interesting spectrum. Crops known to be more salt tolerant include barley, cotton, some oilseeds and many forage plants.

A noteworthy point that has emerged from the extensive studies, conducted at the CSSRI, is that most of the allopolyploid crop species are remarkably more tolerant to both alkali as well as saline soil conditions as compared to their putative diploid progenitors and relatives of lower ploidy levels (Rana, 1978; Rana et al., 1980). Data on response evaluation of large germplasm collections revealed that bread wheats ($2n=6x=42$) were far more tolerant as compared to the durums ($2n=4x=28$) and einkorn wheat ($2n=2x=14$). Among 10 species of *Triticum* whose response to soil salinity and alkalinity stresses was monitored under comparable conditions, hexaploid species *aestivum* was found to be characterised by a distinctly higher tolerance rating and was superior to tetraploid forms, namely, *durum*, *turgidum*, *polonicum* and *carthlicum*. Diploid species *T. monococcum* turned out to be the most sensitive and it failed to flower in alkali soil (pH 9.1) as well as in saline soil (ECe 8 dS m^{-1}). Other allopolyploid species found to be superior in salt stress tolerance included *Brassica juncea* ($2n=36$), *B. napus* ($2n=38$) and *B. carinata* ($2n=34$). In addition, polyploid species *Acacia nilotica* ($2n=52$), *Prosopis juliflora* ($2n=56$), *Zizyphus rotundifolia* ($2n=72$) and *Sporobolus coromandelianus* ($2n=108$) were also found to be highly tolerant to soil salinity/alkalinity conditions.

Further analysis of comparative tolerance ratings of diploids, induced autotetraploids and related allopolyploid forms in *Triticum* led to the conclusion that the superior performance of allopolyploids of this group in stress environments was largely due to favourable genomic interactions and heterotic effects, fixed through polyploidy, rather than to numerical increase in chromosome number. Polyploidy may also be of adaptive significance in the long run in nature because of its capability for throwing up larger genetic variability against environmental selection pressure.

Another noteworthy finding of our studies is that cultivars adapted to alkali soil conditions are also tolerant to salinity but the converse does not seem to hold good particularly in case of crops like barley, that are better adapted to chloride-dominated arid salinity.

VARIATION WITHIN CROPS

Magnitude and hereditability of inter-varietal differences in salt tolerance are important considerations for breeding programmes. Early studies on plant response to salinity, conducted mostly on materials of limited genetic diversity, showed no significant varietal differences within a species and created an impression that there was no appreciable genetic variability within crops to justify breeding efforts for improvement of salt tolerance (Asana and Kale, 1965; Bernstein, 1964, 1975). Beginning with the study of salt tolerance of 60 strains of *Agropyron desertorum* by Dewey (1962), however, convincing evidence has accumulated over the years showing considerable intra-specific genetic variability for such adaptation (Rana, 1978; Shannon, 1978; Epstein et al., 1980; Yeo and Flowers, 1983; Kingsbury and Epstein, 1984; Sayed, 1985).

The CSSRI has made sizeable collections of indigenous cultivars of rice, wheat and barley that are still grown traditionally in different situations of salt-affected soils under little management care. Evaluation of these materials alongwith large germplasm collections of these crops, regarding their tolerance to alkali and saline soil conditions, has led to three important inferences. Firstly, the highest level of salt tolerance was found in indigenous cultivars adapted locally to specific areas of salt-affected soils. Outstandingly salt tolerant wheat materials were identified among red grained Kharchia wheats of Rajasthan's Pali area and Rata wheats of Gujarat's Bhal tract. Two of these selections, namely, KR 375 and KR 387 have been extensively used in hybridisation programmes and also in basic studies on plant adaptation to soil salinity/alkalinity. Data on mineral analysis of plants at the tillering phase, growing in alkali (sodic) soil conditions, revealed that their tolerance was largely due to higher exclusion of Na^+ and better uptake of K^+.

Rice cultivars found to be highly tolerant to soil salinity/alkalinity included Getu, Damodar, Nona Bokra, Nona Sail, Kala Rata, Bhura Rata and Jhona 349. It was noted that the maximum level of tolerance to salinity stress among rice materials was observed in tall and coarse-grained cultivars of longer duration.

Another inference of interest was that the range of genetic variability for tolerance to soil alkalinity/salinity was observed to be greater in wheat as compared to barley accessions screened for this purpose. Though barley cultivars showed a distinct superiority over wheats under conditions of NaCl-dominated arid salinity and limited water availability, yet the magnitude of varietal differences in salt tolerance appeared to be greater among wheats.

GENETIC CONTROL

Plant response to salinity interacts rather strongly with several environmental factors and often diffuses/masks the genotypic differences in tolerance making results of inheritance studies uncertain. A trait showing a distinctly qualitative type of inheritance under controlled experimental conditions, may follow a quantitative type of segregation under the unavoidable heterogeneity of field conditions. It is thus, necessary for genetic studies to develop some suitable experimental set up where salinity/alkalinity levels may be reasonably maintained within defined limits and other major variables may also be controlled. Whereas ion uptake and transport in plants can be monitored with high precision in nutrient cultures raised under controlled conditions, the methodology of working with saline soil culture requires standardisation. Nevertheless, available information shows that tolerance to specific ion effects tends to follow a qualitative type of inheritance governed by single or a few genes with major effects. Tolerance to osmotic effect, on the other hand, appears to follow a quantitative pattern of inheritance.

Genes with major effects on varietal response to soil stress factors have been reported in several crops and they are mostly associated with control of uptake/utilisation of nutrients or exclusion of potentially toxic ions (Devine, 1982; Tal, 1984). Where major gene effects are not clearly discernable, efforts have been made through aneuploid analysis to identify chromosomes contributing significantly towards the observed salt tolerance. For example, chromosomes of the D genome in bread wheats have been reported to contribute significantly to varietal tolerance to alkali, saline and acid soil conditions (Slootmaker, 1974; Rana, 1980; Shah et al., 1987).

A real dilemma for the breeder is to apportion the extent to which yielding ability of a genotype under a given salinity situation depends upon its genetic yield potential *per se* (effective selection for this can be done in favourable environment) and on its adaptation to saline environment, i.e. salinity tolerance advantage (effective selection for this has to be done under stress environment). It appears that a blanket recommendation on this aspect may not be appropriate as suggested by the results reported by different workers (Dewey, 1962; Quisenberry et al., 1980; Rosielle and Hamblin, 1981; Richards, 1983; Kingsbury and Epstein, 1984).

CONVENTIONAL BREEDING EFFORT

Breeding for salinity tolerance began in India during the early part of this century when several rice research stations were established to develop saline and flood resistant varieties for some specific situations. Major attention was focussed upon improvement of locally adapted cultivars of salt-affected and flood-prone areas that form part of present-day Kerala, Tamil Nadu, Andhra Pradesh, Orissa and West Bengal. These pioneering research efforts led to release of some well known selections from locally adapted germplasm collections though the gains in yielding ability were still not very substantial.

Establishment of the Central Soil Salinity Research Institute at Karnal (Haryana) in 1969 by the ICAR provided the necessary fillip to on-going breeding programmes and ushered in a new era by forging suitable linkages to promote exchange of information, ideas and materials among salinity researchers. A stimulating network of purposeful research programmes generated much needed basic information on magnitude and mechanisms of heritable intervarietal differences in tolerance levels of several crops.

Breeding projects for salinity tolerance in India follow two main approaches. Firstly, traditional cultivars of different salt-affected areas are being improved to enhance their yielding ability without lowering their specific adaptations. Besides recombination breeding, rectification of their major shortcomings is also being attempted through induced mutations in combination with conventional breeding methods. Secondly, salinity tolerance is being incorporated into high yielding commercial varieties following usual hybridisation and selection programmes. Excellent donor genotypes for salinity tolerance, having stability of phenotypic expression, have been identified for this purpose in several crops and these are among the most widely used sources of salt tolerance at present sustaining breeding efforts as well as basic studies in many countries.

Although breeding work involving salinity tolerance has been operating at many centres for several decades, yet the pace of progress is slow. Major bottlenecks relate to lack of efficient (and reliable) selection criteria and new specialised breeding methodology designed for this purpose. Another limiting factor, common to nearly all such projects, is the woeful lack of proper size, composition and continuity of the working teams. Paucity of adequate resources and proper motivation is an additional contributing factor. In fact, most aspects of planning, guiding, monitoring and coordination of time-bound linked activities leave much to be desired.

IN VITRO TECHNIQUES

Existing variability for salt tolerance in some crop plants notwithstanding, there is need to develop new sources with higher tolerance levels. In this context, study of salt tolerance at the cellular level and application of tissue culture technique to develop more salt tolerant strains have aroused considerable interest in recent years. Although salt tolerant cell lines were selected in several crop plants, yet the expected gains could not be realised in regeneration of whole plants in most cases indicating the need for further studies. Nevertheless, renewed efforts are underway at many centres to generate new variability for salt tolerance at the cellular level by passage through cell culture (somaclonal variation), mutagenesis and also attempting protoplast fusion with a view to achieving somatic hybridisation between sexually-incompatible species (Widholm, 1978; Stavarek and Rains, 1984; Petolino and Collins, 1984; Ketchum et al., 1987).

Recent exciting developments in the emerging field of biotechnology have also opened up new vistas for *in vitro* breeding for salt tolerance. Using the recombinant DNA technique, a nitrogen-fixing strain of *Rhizobium*, highly sensitive to osmotic stress, was converted into a tolerant type with the help of a broad-host-range plasmid by transferring a DNA segment (of about 10,000 base pairs) from *Escherichia coli* carrying the *osm* gene (s) that led to proline over-production and consequent osmotic tolerance. This technology for genetic manipulation of cellular adaptation to salinity (i. e., osmoregulation) must, however, be suitably integrated with standard plant breeding procedures if this approach is to be

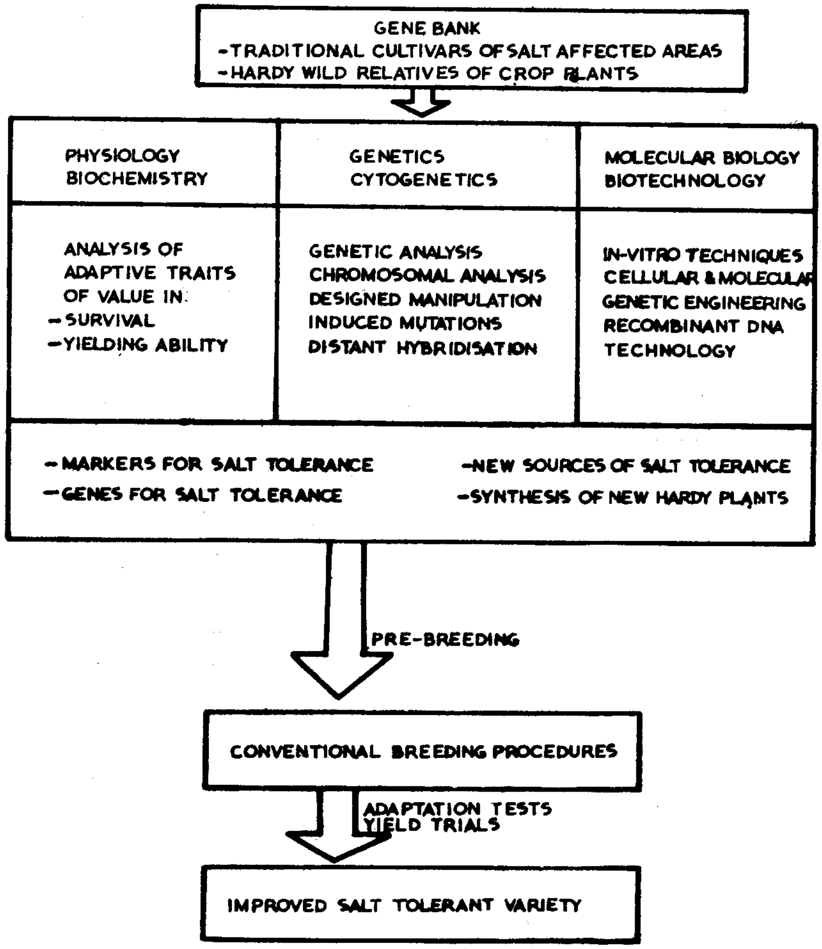

Fig. 1. Genetic Manipulation for Salinity Tolerance—An Integrated Approach.

gainfully employed for genetic improvement of salinity tolerance in crop plants as suggested in Fig. 1.

PRE-BREEDING

Breeders are understandably reluctant to use low-yielding traditional cultivars as donors of salinity tolerance fearing undesirable linkages and apprehending possible disruption of favourable gene combinations brought together in modern high yielding varieties. It is, hence, necessary to undertake "pre-breeding" programmes with the objective of obtaining the desired genes for salinity tolerance in more expressive and agronomically superior genetic backgrounds, in acceptable forms, that may be readily used by the breeders. It is obvious that all promising salt-tolerant lines evolved through wide hybridisation, chromosomal manipulation, tissue culture and genetic engineering approaches, will require to be appropriately processed by adequate pre-breeding work before passing them on to concerned breeding projects.

PROSPECTS

To sum up, development of highly salt tolerant crop varieties having reasonably good yield levels is the basic requirement for evolving low cost-low risk technology for the benefit of marginal farmers owning salt-affected lands. The job of breeding such varieties is, however, more difficult than that of developing varieties for favourable and assured environments. These projects need, therefore, more specialised facilities, greater technical skill, better planning, more effective coordination and more resources. Progress in combining superior salt tolerance with good yielding ability has been rather limited so far owing largely to non-availability of reliable markers for salt tolerance and the breeder's difficulty in determining the extent to which yield obtained under a particular saline environment (of his target area) depends upon traits that contribute to yield *per se* and on traits that improve establishment and survival under such conditions. Optimism in this challenging line of work stems, however, from some notable advances already made in some crops such as rice, barley, wheat, tomato and forages (Ramage, 1982; Kingsbury and Epstein, 1984; Jones and Qualset, 1984; Epstein, 1985; Rana, 1986).

Wide crosses are likely to play a key role in these projects since many wild relatives of the cultivated plants, growing naturally in salt-affected areas over long periods, are reported to be highly salt tolerant (Tsitsin, 1962; Stalker, 1980). For example, *Elytrigia* (= *Agropyron*) *elongatum* ($2n = 2x = 14$) and *E. pontica* ($2n = 10x = 70$) have been reported to survive salt concentrations as high as 1.5 times seawater (Dewey, 1960; Shannon, 1978; McGuire and Dvorak, 1981) and are being exploited for enhancing adaptation of wheat cultivars to saline environment (Dvorak et al., 1985; Storey et al., 1985).

Prospects for successful application of the tissue (cell) culture technique in this field also appear to be bright despite the obvious limitation of *in vitro* screening and selection for salt tolerance namely, that it can only identify variants that are expressed at the cellular level. Thus, although certain traits such as tolerances to salts, soil pH, heavy metals and herbicides may prove accessible through *in vitro* approach, others like yield and flowering seem to be beyond the reach of the somatic cell geneticists, at least for the present. The developmental complexity of crop plants makes this restriction appear to be rather severe at the moment but it could well be due to our poor understanding of its molecular and cellular bases. Nevertheless, observation of significant differences in salt tolerance at the cellular level does point out a possibility of improving salinity tolerance levels by hopefully combining it with the tolerance marked in reproductive phase. Data presented in Table 2 illustrate this point. Salinity tolerance ratings computed from response curves showed that wheat variety WH 157 and barley variety BG 25 were significantly more tolerant than others at the callus level whereas

TABLE 2. SALINITY TOLERANCE RATINGS OF SOME WHEAT AND BARLEY GENOTYPES BASED ON RESPONSE OF THEIR CALLI AND WHOLE PLANTS

Genotypes	Calli	Whole plants
	Dry weight	Grain yield
Wheats :		
HD 2009	0.81	0.86
WH 157	1.23	1.03
KR 375	1.10	1.31
Barleys :		
IE 65	0.43	0.58
BG 25	1.25	1.16
Ratna	0.84	1.26

wheat line KR 387 and barley variety Ratna were distinctly superior at the whole plant level (yield). More genetic variability at the cellular level may also be induced through mutagenic treatments and desired phenotypes can be selected directly by defining culture conditions that favour growth of more salt tolerant cells and discriminate against growth of 'normal' cells. A current bottleneck limiting the pace of such researches is the ability of cells to retain their adaptability to regenerate plants through the expectedly prolonged periods in culture. Such sustained morphogenetic competence tends, however, to be the exception rather than the rule among our major crop plants (Chaleff, 1983). Another area of promising researches on this subject relates to study of gene expression in salt-stressed (also heat shocks, etc.) tissue to differentiate salt-stress specific responses from general responses. Work progressing at J. L. Key's laboratory at the University of Georgia has shown that a group of homologous RNAs (and stress-induced proteins) are induced in soybean following several different types of stresses.

Development of highly salt tolerant crop varieties having reasonably good yield levels is the key factor in evolving low cost-low risk technology needed for marginal farmers owning salt-affected lands. Urgency for developing such technology is more for the developing countries. They also happen to be the centres of origin of our major crops and contain enormous genetic diversity useful for evolving varieties better adapted to local ecosystems and adverse soil conditions. Breeding for higher salt tolerance is thus a challenge, and also an opportunity, for breeders and salinity researchers in developing countries. They are in the best position for making advances in this emerging field as they have at their disposal enormous variability in local landraces adapted to specific situations of ecological stresses.

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