Prospects for Saline Agriculture in Pakistan: Today and Tomorrow

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Abstract: A brief description of soil salinity has been presented with reference to Pakistan along with biological approach for controlling this problem. Cultivation of Kallar grass (Leptochloa fusca) at saline/alkaline soils has been specially mentioned in this connection. After giving an account of the mechanism of salt tolerance in plant some modern techniques used have been mentioned in this connection. Extant of salt tolerance investigated through hydroponic culture has been listed for some plants.

Keywords: Salt tolerance, Kallar grass, Plant breeding, Recombination DNA technology.

Introduction

Salinity, sodicity and water logging are major problems of agriculture of Pakistan. At present around 6.5 million hectares of land is salt affected. This salinization is mainly due to lack of drainage of groundwater and a rising water table caused by extensive seepage from the rivers and the irrigation systems. Complete solutions of this problem would entail an effective drainage system throughout the Indus Basin. That would not only require inputs of capital and energy on a scale far beyond available national resources but would also need decades to complete.

In view of this situation, scientists at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad had been working on the basis that soils are not just a mass of dead chemicals but is a living system harbouring numerous chemical and biological processes and is in constant interaction with several environmental factors. The saline-sodic soils have an excess of sodium, are impermeable to water, have little or no organic matter and are biologically almost dead. Based on these assumptions, Sandhu and Malik (1975) proposed a plant succession on such soils starting from highly salt tolerant plants followed by lesser salt tolerant plants. This strategy has been termed as Biological Approach for utilization of salt affected soils (Malik 1978). In this scheme, Leptochloa fusca (Kallar grass), being highly salt tolerant to salinity (Sandhu et.al. 1981) and sodicity (Ahmad et.al. 1979) is used as primary colonizer for plant establishment and biomass production on saline lands. Soil conditions also improve in the process and less salt tolerant plants can be introduced.

Introduction of a salt tolerant crop will provide a green cover and will improve the environment for biological activity, increase organic matter and will help fertility. The penetrating roots will provide crevices for downward movement of water and thus help leaching of salts from the surface. The plant growth will also result in higher carbon dioxide levels, and would thus create acidic conditions in the soil that would dissolve the insoluble calcium carbonate and will help exchange of sodium with calcium ions on the soil complex. Further, the biomass produced could also be used as green manure which will quicken the lowering of pH and result in further release of ionic calcium. The soil structure, its permeability, its biological activity and fertility cold thus be restored and with extra irrigation the surface salts could be leached down (Malik et.al. 1986)

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A complete amelioration of the deleterious soil effect can be achieved if good irrigation water for leaching the salts is available. However, irrigation is already in a short supply for existing arable lands in Pakistan and therefore its use for reclaiming the salt affected wastelands is not feasible. In order to overcome this problem, brackish underground water has been used for leaching the salts in the above described biological approach. The chemical and physical properties of the saline sodic soils where Kallar grass was grown for different periods were monitored. It was shown that the relative hydraulic conductivity increased which resulted in an accelerated leaching of salts downward resulting in removal of salts form the top soil layer essential for plant growth (Akhtar et.al. 1988)

In order to effectively implement this biological approach, work has been carried out on the following aspects:

- Screening of plants for salt tolerance.
- Mechanism of salt tolerance.
- Development of salt tolerant variation
- Conventional plant breeding
- Recombinant DNA technology
- Utilization of biomass on saline lands.

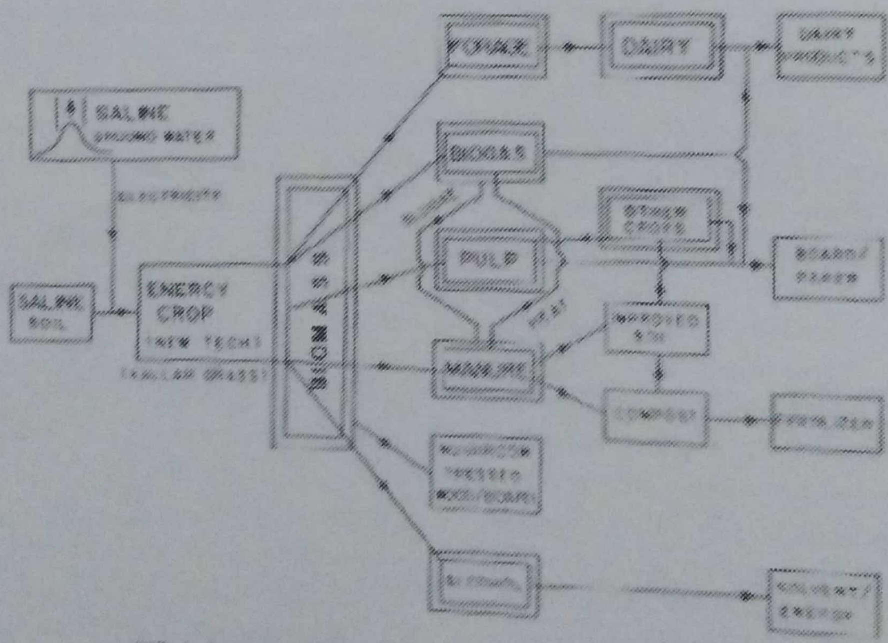

FIG. 1. A schematic presentation of the possible uses of Kallar grass

Screening of plants for salt tolerance

The plants are not only known to grow in all kinds of extreme environments but also have inherent ability to adapt to varying degrees to such stress. Therefore the best strategy is first to look for natural ability of plants to tolerate such abiotic stresses like salinity. In order to accumulate this information large number of germplasm of different plant species collected from Pakistan and elsewhere have been screened using a gravel culture hydroponic method (Qureshi 1977). The salt tolerance limits were calculated on the basis of 50% reduction in the biomass yield as compared to the control. An up to date list of plant species screened so far is presented in Table I. The plants are listed in their decreasing order of salt tolerance. These include forage crops, legumes, different grasses and some fast growing trees (Niazi et.al. 1985, 1987; Mahmood et.al.1986, NIAB 1987). The screening of all the germplasm is quite time consuming and laborious as all the plant species tested were grown to maturity or flowering before determining their salt tolerance. However such information is useful while devising strategies for utilizing salt affected areas and also for introducing salt tolerance to other crops using conventional or modern biotechnological methods.

Majority of the plant species screened so far have also been tested in the field at the Biosaline Research Station of NIAB near Lahore. However among all the plants screened, *Leptochloa fusca* (Kallar grass) has been selected as the primary colonizer of saline lands for its various properties including salt tolerance. It is a perennial grass having C-4 photosynthetic pathway (Zafar and Malik 1984) and having highly efficient associative nitrogen fixation in its rhizosphere (Malik et.al. 1988; Malik and Bilal 1988) with its annual biomass yield as high as 50 tons/ha. This grass has therefore been used as a model lignocellulosic substrate that can be produced on salt affected wastelands and converted by various biotechnological procedures into value added products. Such possible uses of Kallar grass have been presented in Figure. 1.

Mechanism of salt tolerance

For a successful strategy for developing salt tolerant plant species, an understanding of the basis of osmoregulation is essential (Mccue and Hanson 1990). It is generally

Part of this table is taken from "15 years of NIAB", a report published by Nuclear Institute for Agriculture and Biology 1987. This table is based on published material referred to in the text believed that there is no universal mechanism of salt tolerance. However. Some of the mechanisms so far encountered are (i) curtailment of Na^+ influx and prevention of intracellular Na accumulation that reduce the need to pump out excess Na^+ and converse energy.

Table. 1. Salt Tolerance Of Different Species/Varieties Carried 0ut Under Controlled Hydroponic Condition. All Plants Were Grown To Maturity/Flowering

Species/variety	Salt tolerance (mS/cm)
Suaeda fruticosa	48.0
Kochia indica	38.0
Atriplex amnicola	33.0
Acacia combagei	27.7
Atriplex lentiformis	23.0
Atriplex undulata	22.5
Atriplex crassifolia	22.5
Leptochloa fusca (Kallar grass)	22.0
Sporobolus arabicus	21.7
Cynodon dactylon (Tift 78)	21.0
Brassica napus (Gobhi sarson)	19.5
Beta vulgaris (Fodder beet)	19.0
Hordeum vulgar (barley) 6 cultivars	19.5
Sorghum vulgare (JS-263)	16.7
Sorghum vulgare (JS-1)	16.5
Acacia calcicola	16.5
Panicum antidotale	16.0
Sorghum valgare (Japani millet)	15.0
Pholypogon monspeliensis	13.7
Cynodon doctylon	13.2
Sesbania aculeata (Dhancha)	13.0
Hasawi rushad	12.5
Leucaena leucocephala (Ipil-Ipil)	12.4
Medicago sativa (Lucerne Hajazi)	12,2
Sesbania rostrata	12.0
Macroptilium atropurpureum (Siratro)	12.0
Lolium multiflorum (Italian rey grass)	11.2
Echinochloa colonum (Swank)	11.2
Acacia kempeana	11.0
Dichanthium annulatum	11.0
Acacia aneura	5.0
Acacia cunnighamii	9.4
Acacia holosericea	9.0
Desmostachya bipinnata	9.0
Panicum maxium (N-S-I)	9.0
Panicum maxium (Exotic)	8.5
Sorghum halepene	7.0

(ii) Accumulation of internal osmoticum in the form of inorganic ions such as K^+ or organic solutes such as glycerol, sucrose, trehalose, proline, glutamate, glutamine or glycine solutes such as glycerol, sucrose, trehalose, proline, glutamate,Glutamine or glycine betaine and

(iii) metabolic adjustments to tune the cellular activities to function at higher internal osmoticum. All these mechanisms imply modifications of the synthesis of cell proteins to facilitate osmotic adaptation. Recently Maathuis and Antmann (1999) have reviewed the role K^+/Na^+ ration in salt tolerance. Several proteins have been characterized that play prominent roles in the regulation of K^+ and /or Na^+ fluxes.

Detailed investigations have been made on the mechanism of salt tolerance in Kallar grass (Malik et.al. 1986). It showed high uptake of salts (Sandhu et.al. 1981; Bhatti et.al. 1985; Abdullah et.al. 1985) and there is no restriction on transport of Na^+ and $C1^-$ from roots to leave. Increased concentration of Na^+ and $C1^-$ in leaves and roots did not affect plant growth and no toxic symptoms were observed in the leaves. Sandhu et al. (1981) observed the accumulation of glycine betaine and proline in the leaves/roots, which act as compatible cytoplasmic solutes. Recently Aziz et al. (1993) studied salt response proteins of two ecotypes of Kallar grass and Atriplex and probed with antibodies raised against some of the slat induced protein of *Klebsiella sp*. NIAB-1 a bacterium isolated from roots of kallar grass. These results indicated presence of common epitopes among the salt responsive proteins.

Development of salt tolerant plants

Plant breeding

The conventional approach for developing new plant varieties is through selection and breeding for high yield, disease resistance and other traits. The breeding programme for salt tolerance run into difficulty because of lack of basic understanding of the mechanisms of salt tolerance which is a polygenic character governed at levels of organization ranging from subcellular to organism. Most of the efforts in plant breeding for salt tolerance have been carried out in economic crops like wheat and rice (WYN Jones et.al. 1990; Brar et.al. 1986). However these efforts are constrained by the fact that many of the wild species for example wild rice which show some salt tolerance have no crossability with cultivated rice (Sitch et.al. 1990). Moreover non-availability of any phenotypic or biochemical markers for salt tolerance makes the conventional plant breeding quite difficult. Efforts have also been made to use radiation-induced mutation to crate genetic variability for selection of salt tolerance (Sajjad et.al. 1984) but desired results have not been obtained.

Invitro Technologies

The application of biotechnology to the genetic improvement of plant tolerance to salt stress offers exciting possibilities and in addition provides basic information regarding the biochemical and physiological mechanisms related to salt tolerance. One of such techniques which overcomes the problems of crossability of two species, is somatic hybridization through protoplasts fusion of two different plant species, one of which is salt tolerant. The hybrid cells could be selected by imposition of salt stress. The important step in such an approach is the availability of a method to regenerate the hybrid into whole plant. Some success has been obtained in several plant species namely brassica, potato, tobacco, alpha alpha, petunia, citrus etc. (Climelius et.al. 1991). Such an approach is now being applied to more important food crops such as rice and sugarcane. In recent years, somatic hybridization through protoplast fusion in rice has successfully been achieved by (Tereda et al. 1987); (Hayashi et al. 1988); (Finch et al. 1990). Plant regeneration through tissue culture has been achieved in basmati rice (Zafar et.al 1992).

The wild species represent an important reservoir of genetic diversity and are a source of genes controlling natural resistance to biotic and abiotic stresses and other characters useful to rice breeders. Protoplast fusion coupled with an efficient screening protocol might be a practical way to transfer polygenic traits. It is a valuable complement to established plant breeding methods.

Recombinant DNA technology

With the recent developments in molecular biology it is now possible to transfer genes from prokaryotes to eukaryotes. A number of genes have been found to contribute to osmotic adaptation in enteric bacteria. Prominent among these are Kdp A-E required for K^+ uptake (Csonka et.al. 1989; Epstein et.al. 1986); ProU and proP required for transport of proline and glycine betaine; pro ABC required for synthesis of B proline, otsA and otsB required for synthesis of trehalose and betABT required for transport of choline and synthesis of glycine betaine from choline (May et.al. 1986). Recently Winicov (1998) has reviewed the new molecular approaches to improving salt tolerance in crop plants. Holmbers and Bulow (1998) reviewed the mechanism of in addition to these genes involved in osmoregulation, a plasmid pNIA.B 1 has been discovered in *Klebsiella salinarium* a mail bacteria, which harbors genes for salt tolerance (Qureshi et.al. 1990). This observation has been confirmed by genetic transformation of pNIAB-1 to *E. coli* K12 and *K. pnumoniae* M5A1. This plasmid has been characterized and has been shown to carry a 1.9 kb fragment which codes for glycine betaine transport (Qureshi et.al.). This fragment is now being used to transform rice using pACT1 D vector after placing the fragment under rice Actin promoter for expression.

Utilization of biomass

One of the main facets of the biological approach is the economic utilization of biomass produced on saline lands using brackish underground water. One of the source of biomass in Leptochloa fusca (Kallar grass) which has been extensively studied (Malik et.al. 1986). Some of the uses of this grass are presented in *Fig. 1.* Its use as fodder is quite well established and its effect on livestock nutrition has been studied (Khanum et.al. 1986). The conversion of this material to compost has also been accomplished (Mahmood et.al 1987)..

Photosynthesis is still the most efficient method for converting solar energy to chemical energy. Kallar grass has been used as a model biomass composed of lignin, cellulose, hemicellulose etc which is common to all such biomass. Using various biotechnological methods it is now possible to convert this biomass into value added products thus making the biomass production of saline soil an economic proposition.

Conclusions

The biological approach for economic utilization of salt affected wastelands has become a reality as many national agencies and international organizations are keenly pursuing it because of its sustainable and environment friendly nature. It not only improves the general ecology of the area but in return provides farmers with economic benefits [8]. In order to improve this approach and derive maximum benefits, continuous input from scientific research both in basic and fields is essential.

Refrences

Abdullah, M., Qureshi, (1985) Rochloa fusca to various typs of substrate sainities. In: Prospects or Biosaline Research. Ahmad R and San Pietro (Eds.) Plenum Press, New York .

Ahmad, Z., Saleem, M., Sanmdhu, G.R., Qureshi, R.H., (1979) Sodicity effects on growth and chemical composition of *Diplachne fusca* (Kallar grass). Pak. J. Bot. 11:123-128.

Akhtar, J., Waheed, R.A., Niazi, M.L.K., Malik, K.A., Naqvi, S.H.M., (1988) Moisture properties of a saline sodic soil as affected by growing Kallar grass using brackish water. Reclamation and Revegetation Res.

Anonymous (1987) Fifteen Years of NIAB , A Report. Nuclear Institute of Agriculture and Biology, Faisalabad.

Aziz N. A., Ali S. H., Ahmad N., Qureshi J. A. and Malik K. A. (1993). Comparison of salt stress responsive proteins in *Atriplex amnicola* antibodies and two ecotypes of *Leptochloa fusca* (L.) Kunth. Pak. J. Botany, 25(1): 29-39.

Bhatti S.A., Sarwar, G., Tahir, M., (1985) Characteristics of N+ and K+ absorption in Leptochloa fusca (L) Kunth. In Prospects or biosaline rsearch (Ahmad R and San Pietro A (Eds), Plenum Press, New York.

Brar, D.S., Khush, G.S., (1986) Wide hybridization and chromosome manipulation in cereals. In: Handbook of plant cell culture, vol: 4. Techniques and Applications. D A Evans et al (Eds), Macmillan Pub. Co. New York.

Csonka, L.N. (1989) Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev. 53:

Epstein, W., (1986) Osmoregulation by potassium transport in *E coli.* FEMS. Microbial. Rev. 39: 73-78.

Finch, R.P., Slamet, I.H., Cocking, E.C (1990) Production of heterokaryons by the fusion of mesophile protoplast of *Porteresia coarctata* and cell suspension derived protoplast of Oryza sativa. A new approach to somatic hybridization in rice. J. Plant Physiol. 136: 589-592.

Glimelius, K., (1991) Gene transfer via somatic hybridization in plants. TIBTECH 9:24-30

Hayashi, Y., Kyozaka, J., Shimamoto, K. (1988) Hybrids of rice (*Oryza sativa* L.) and wild oryza species obtained by cel,l fusion Mol. Gen. Genet. 214: 6-10

Holmberg N and Bulow L (1998) Improving stress tolerance in plants by gene transfer. Trends in plant science 3(2): 61-66

Khanum S.A., Ali, M., Naqvi, S.M.H.(1986) Effect of feeding salt tolerant grasses on reproductive efficiency of dwarf goats. Paper presented at International Symposium on the use of Nuclear Techniques in Studies of Animal Production and Health in different Environments, IAEA Vienna, Austria, 26-27

Maathius F.J.M. and Amtmann A., (1999) K^+ Nutrition and Na^+ Toxicity: The Basis of Cellular ka+/Na+ Ratios. Annals of Botany 84: 123-133

Mahmood K. and Malik K.A. (1986) Salt tolerant studies on *Atriplex rhagodioides.* Environmental and Experimetal Botany 27:119-125

Mahmood, T., Azam, F., Malik, K.A. (1987) Salt tolerance studies in different cultivars of barley. Pak. J. Bot. 19:

Malik, K.A., (1978) Biological methods of reclamation of salt affected soils. In: Technology for Increasing Food Production. Proc. 2[nd] FAO/IAEA Seminar on Field Food Crops in Africa and New East, Lahore Pakistan

Malik, K.A., Bilal, R., (1988) Survivals and colonization of inoculated bacteria in Kallar grass rhizosphere and quantification of N2-fixation. Plant and Soil, 110:

Malik K.A., Aslam, Z., Naqvi S.H.M., (1986) Kallar grass - A plant for saline land. Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan

Malik, K.A., Bilal, R., Azam, F., Sajjad, M.I., (1988) Quantification of N_2-fixation and survival of inoculated diazotrophs associated with roots of Kallar grass. Plant and Soil 108: 43-51.

May, G., Faatz, E., Villarejo, M., Brembner, E., (1986) Binding protein dependent transport of glycine betaine and its osmotic regulation in *Escherichia coli* K12. Mol. Geb. Genet. 205: 225-233

Mccue U.F.and Hanson A.D. (1990) Drought and salt tolerance: towards understanding and application. TIBTECH 8:358-362

Niazi M.L.K., Haq M.I. and Malik K.A.(1985) Salt tolerance studies on Ipil Ipil *(Leucaena leucocephela* L.) Pak. J. Bot 17:43-47

Niazi M.L.K., Mahmood K. and Malik K.A. (1987) Salt tolerance studies in different cultivars of barley. Pak. J. Bot. 19:17-27

Qureshi, J.A., Malik, K.A., (1990) Evidence for a plasmid conferring salt-tolerance in the plant-root associated, diazotroph Klebsiella sp. Biotechnology Letters, Vol. 12(2)

Qureshi, J.A., Ali R., Shah W.A., Ahmad N, Khattak K.F. & Malik K.A. (1993). Hypersaline-stress Protection in Organisms Found in Saline-Soils. *In:* Proceedings of International Symposium on Biotechnology for Sustainable Development, Dec. 15-20, 1993. Eds. Kauser A. Malik, Anwar Naseem & A.M. Khalid, NIBGE, Faisalabad, Pakistan

Qureshi, R.H., Salim, M., Aslam, Z., Sandhu, G.R. (1977) An improved gravel culture technique for salt tolerance studies in plants. Pak. J. Agric. Sci, 14: 11-18.

Sajjad, M.S., (1984) Breeding for salt tolerance in rice strains Intl. Rice Res. Newsletter 9:

Sandhu, G.R. and Malik, K.A., (1975) Plant succession-A key to the utilization of saline soils. The nucleus, 12: 35-38.

Sandhu, G.R., Aslam, Z., Saleem, M., Sattar, A., Qureshi, R.H., Ahmad, N., Wynjones, R.G., (1981) The effect of salinity on the yield and composition of Diplachne fusca (Kallar grass). Plant, Cell and Environment 4

Sitch, L.A., (1990) Incompatibility barries oprating in crosses of Oryza sativa with related species and genera. In: genetic manipulations in Plant Improvement. Gustafson JP (ed).

Tereda R., Kyozaka, J.,Nishibayashi, S., Shimamoto, J.K., (1987) Plant regeneration from somatic hyubrids of rice (Oryza sativa L.) and barnyard grass (E. chinochloa oryzicola vasing) Mol. Gen. Genet. 210

Winicov I, (1998) New Molecular Approaches to Improving Salt Tolerance in Crop Plants. Annals of Botany 82: 703-710.

Wyn Jones, R.G., Storey, R., Leigh, R.A., Ahmad, N., Pollard, A., (1977) A hypothesis in cytoplasmic osmoregulation. In: Regulation of cell membrane activities in plants. Marre e and Ciferri O (Eds) Amsterdam: 121-136

Zafar, Y., Malik, K.A., (1984) Photosynthetic system of Leptochloa fusca, Pak. J. Bot. 16(2):

Zafar, Y., Wajid, A., Malik, K.A., Gamborg, *O.L.*, (1992) Establishment of regenerating calli and cell suspension line of basmati rice (Oryza sativa L. cv. B-370). Pak. J. Bot. 24(1): 67-71