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Invited Paper

CROP PRODUCTION IN SALT AFFECTED SOILS: A BIOLOGICAL APPROACH

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Abstract

CROP PRODUCTION IN SALT AFFECTED SOILS: A BIOLOGICAL APPROACH.

Plants are susceptible to deleterious effects of various abiotic and biotic stresses, thus grossly affecting the growth and productivity. Amongst the abiotic stresses, soil salinity is most significant and prevalent in both developed and developing countries. As a consequences, good productive lands are being desertified at a very high pace. To combat this problem, various approaches involving soil management and drainage are underway but with little success. It seems that a durable solution of the salinity and water-logging problems may take a long time and we may have to learn to live with salinity and to find other ways to utilize the affected lands fruitfully. A possible approach could be to tailor plants to suit the deleterious environment. The saline-sodic soils have excess of sodium, are impermeable, have little or no organic matter and are biologically almost dead. Introduction of a salt tolerant crop will provide a green cover and will improve the environment for biological activity, increase organic matter and will improve the soil fertility. The plant growth will result in higher carbon dioxide levels, and would thus create acidic conditions in the soil which would dissolve the insoluble calcium carbonate and will help exchange sodium with calcium ions on the soil complex. The biomass produced could be used directly as fodder or by the use of biotechnological and other procedures it could be converted into other value added products. However, in order to tailor plants to suit these deleterious environments, acquisition of better understanding of the biochemical and genetic aspects of salt tolerance at the cellular/molecular level is essential. For this purpose model systems have been carefully selected to carry out fundamental basic research that elucidates and identifies the major factors that confer salt tolerance in a living system. With the development of modern biotechnological methods it is now possible to introduce any foreign genetic material known to confer salt tolerance into crop plants. Some of the approaches and results obtained are being discussed.

1. INTRODUCTION

Crops are susceptible to deleterious affects of various biotic and abiotic stresses. Among the biotic stresses, the plant diseases caused by virus, bacteria, fungi, nematodes, insect and pest are the most prevalent factors affecting crop productivity. However the abiotic stresses which include drought, salinity, water logging, temperature and soil chemical and physical stresses are most widespread and are a major impediment in increasing crop productivity.

Amongst the abiotic stresses, soil salinity is most significant and wide spread cause of reduction in crop yields both in developed and developing countries especially in the irrigated areas. Most major irrigation schemes through out the world suffer to some degree from the effects of salinity. There are no accurate statistics available as to the extent of this damage. FAO and UNESCO [1] have estimated that about one half of all existing irrigation systems (totalling about 250 mha) are seriously affected by salinity and water logging and that 10 mha of irrigated land are abandoned annually.

In Pakistan, this hazard assumes more serious proportions as we have one of the world's largest irrigation system. According to one estimate there are 14 million acres of salt affected land out of which 3 million acres are presently in the canal commanded area [3]. The remaining 11 million acres now termed as salt affected wastelands also have a history of being productive and fertile in the past. This salinity has adversely affected the soil chemical and physical properties making it unfit for conventional crop production.

2. CAUSES OF SALINIZATION

The main source of salts found in saline soils can be the parent material, the irrigation water, the under ground water or excessive use of fertilizers which may be the case in the developed countries. The salt composition of the soil water influences the composition of cations on the exchange complex of the soil colloids and jointly salinity level and exchangeable cation composition influences soil permeability and other physical properties [2]. All irrigation waters contain some salt which concentrates in the root zone because water and not all the salts, are taken up by crops. Accordingly to an estimate by Rhoades and Loveday [2] each application of 100 mm depth of water containing as little as 500 mg salts/L adds 500 kg of salt to each hectare of irrigated land; this salt concentration will progressively increase over time and irrigation unless it is removed through leaching and drainage. Salts within the root zone may be redistributed towards the soil surface through upward flux of water driven by evaporation in the absence of net downward flux through leaching and drainage.

In Pakistan the salinity is mainly due to the seepage of water from the rivers and irrigation canals constituting the world's largest irrigation system. Seepage and lack of drainage system have resulted in rise in water table and accumulation of salts in the soil profile. To combat this problem various approaches involving soil management and drainage are underway but with little success. It seems that a durable solution of the salinity and water-logging problems may take a long time and we may have to learn to live with salinity and will have to find other ways to utilize the affected lands fruitfully.

3. BIOLOGICAL APPROACH

A possible approach could be to tailor plants to suit the deleterious environment, Sandhu and Malik [4] proposed plant succession scheme for utilization of salt affected soils. In this scheme, *Leptochloa fusca* (Kallar grass), being highly salt tolerant to salinity [5] and sodicity [6] 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.

Such an approach is essentially based on the rationale that the soil is not just a mass of dead chemicals but is a living system undergoing numerous chemical and biological reactions and is in constant interaction with numerous environmental factors. The saline-sodic soils have excess of sodium, are impermeable, have little or no organic matter and are biologically almost dead. 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 which 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 could thus be restored and with extra irrigation the surface salts could be leached down [7,8].

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 and therefore its use for reclaiming the salt affected waste lands 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 soil where Kallar grass was grown for different periods were monitored. It was shown that the relative hydraulic conductivity increased which resulted in accelerated leaching of salts downward resulting in removal of salts from the top soil layer essential for plant growth [9].

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
  - breeding
  - protoplast fusion / Somaclonal variation
  - Recombinant DNA technology
- Utilization of biomass on saline lands.

3.1. 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 a large number of germplasm of different plant species collected from Pakistan and elsewhere have been screened using a gravel culture hydroponic method [36]. The salt tolerance limits were calculated on the basis of 50% reduction in the biomass yield as compared to the control. An uptodate 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 [5,12,13,14,37]. 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 an 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 [10] and having highly efficient associative nitrogen fixation in its rhizosphere [11,15,16,17] 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 Fig. 1.

3.2. Mechanism of salt tolerance

For a successful strategy for developing salt tolerant plant species, an understanding of the basis of osmoregulation is essential [19]. It is generally believed that there is no universal mechanism

TABLE I. SALT TOLERANCE OF DIFFERENT SPECIES/VARIETIES CARRIED OUT 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 cambagei	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 vulgare (*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 vulgare (*Japani millet*)	15.0
Polypogon monspeliensis	13.7
Cynodon dactylon	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	9.5
Acacia cunnighamii	9.4
Acacia holosericea	9.0
Desmostachya bipinnata	9.0
Panicum maximum (*N-S-1*)	9.0
Panicum maximum (*Exotic*)	8.5
Sorghum halepense	7.0

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.

of salt tolerance. However. some of the mechanisms so far encountered are (i) curtailment of Na^+ influx and prevention of intracellular Na accumulation which reduce the need to pump out excess Na^+ and conserve energy. (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 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 adaption.

Detailed investigations have been made on the mechanism of salt tolerance in this grass [8]. It showed high uptake of salts [5,38,39] and there is no restriction on transport of Na^+ and Cl^- from roots to leaves. Increased concentration of Na^+ and Cl^- in leaves and roots did not affect plant growth

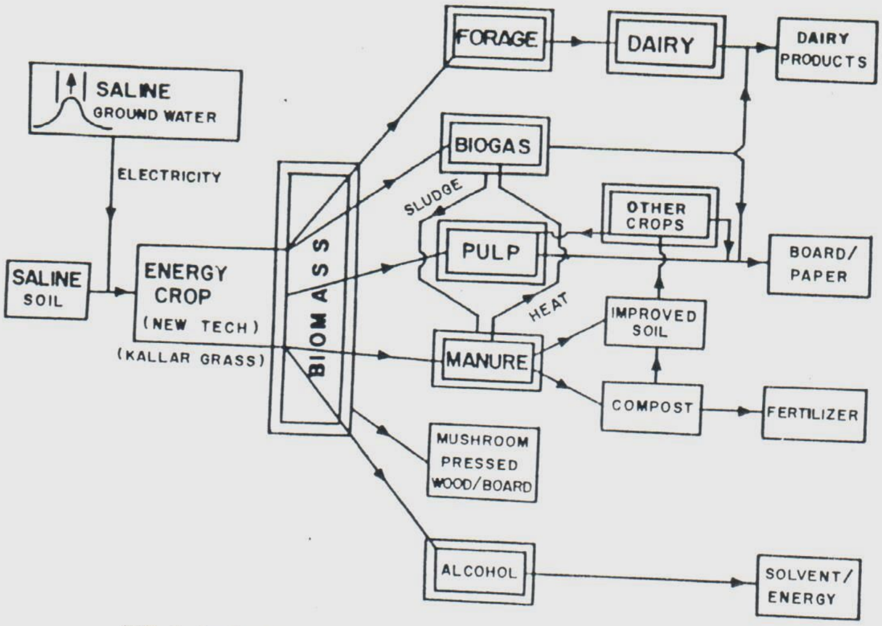

FIG. 1. A schematic presentation of the possible uses of Kallar grass.

and no toxic symptoms were observed in the leaves. Sandhu et al. [5] observed the accumulation of glycine betaine and proline in the leaves/roots which act as compatible cytoplasmic solutes. Recently Aziz et al.[43] studied salt response protein of 2 ecotypes of Kallar grass and *Atriplex* and probed with antibodies raised against some of the salt induced protein of *Klebsiella sp. NIAB-1*. These results indicated presence of common epitopes among the salt responsive proteins.

3.3. Development of salt tolerant plants

3.3.1. 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 organismic. Most of the efforts in plant breeding for salt tolerance have been carried out in economic crops like wheat and rice [34,35]. 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 [33]. 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 [32] but desired results have not been obtained.

3.3.2. Protoplast fusion

The application of biotechnology to the genetic imrovement of plant tolerance to salt stress offers exciting possibilities and in addition provides basic information regarding the biochemical and

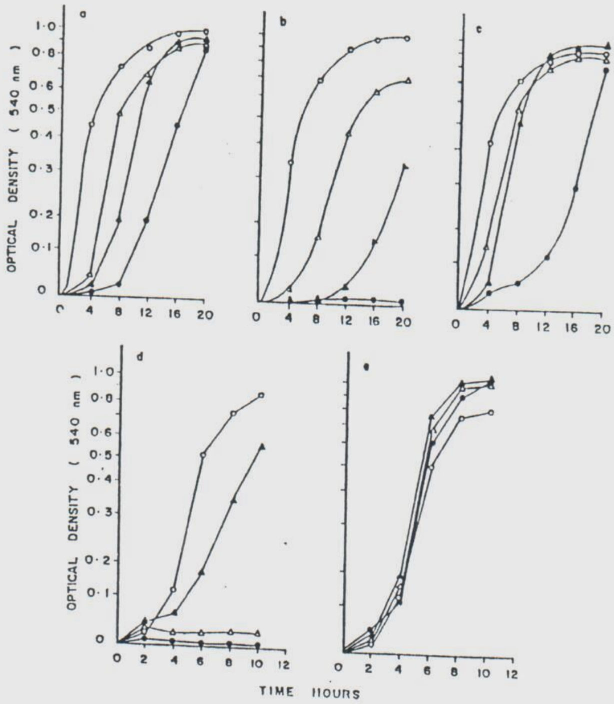

FIG. 2. Effect of NaCl concentration adjusted at pH 8.0 on the growth of (a) Klebsiella NIAB-1 (b) M5a1, (c) M5a1 harboring pNIAB-1, (d) MV10 and (e) MV10 harboring pNIAB-1. The optical density of the cultures is plotted as a function of incubation time. Open circles represent control; closed circles 1M NaCl; closed triangle 0.8M NaCl and open triangle 0.6M NaCl [40].

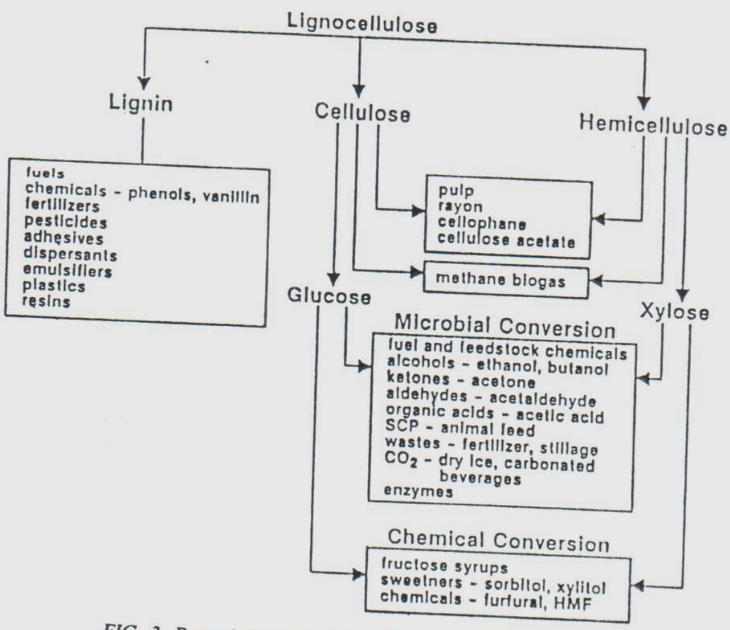

FIG. 3. Potential by-products of lignocellulosic biomass.

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. [18]. 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. [23]; Hayashi et al. [22]; Finch et al. [20,21]. Plant regeneration through tissue culture has been achieved in basmati rice [24]. Protoplast technology for several other cultivated indica rice species and few wild rice species are being developed for somatic hybridization studies at NIBGE. 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 the only practical way to transfer polygenic traits. It is a valuable complement to established plant breeding methods.

3.3.3. 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 [25,26]; 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 [31].

In addition to these genes involved in osmoregulation, a plasmid pNIAB-1 has been discovered in *Klebsiella salinarium* which harbors genes for salt tolerance [40]. This observation has been confirmed by genetic transformation of pNIAB-1 to *E. coli* K12 and *K. pnumoniae* M5A1 (Fig. 2). This plasmid has been characterized and has been shown to carry a 1.9 kb fragment which codes for glycine betaine transport [27]. This fragment is now being used to transform rice using pACT1-D vector after placing the fragment under rice actin promoter for expression.

3.4. 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 [8]. Some of the uses of this grass are already presented in Fig. 1. Its use as fodder is quite well established and its effect on livestock nutrition has been studied [41]. The conversion of this material to compost has also been accomplished [42]).

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.

Some of the possible uses of lignocellulosic biomass are presented in Fig. 3. Among these the most attractive options are conversion of biomass to alcohol and methane gas. Such process will add directly to the economy of the country by alleviating energy shortages which are prevalent in all the developing countries. Several studies have been carried out to show the feasibility of both aerobic and anaerobic conversion of such lignocellulosic biomass to alcohol and methane gas [29,30]. Optimization of the process has been carried out and in order to further simplify the process, some of the cellulase genes have been cloned into *Saccharomyces cervisiae* [28]. In addition, several microorganisms have been isolated and characterized which can perform various function for upgrading the economic value of the biomass produced on salt affected soils.

4. 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.

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