Developing Cash Crops from Halophytes

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Section 1. Halophytes, New Salt Tolerant Crops

Abstract. A large number of halophytes are already exploited commercially (see list published as annex in Hamdy et al ed. 1999). There are many other species from the list recently published by Menzel and Lieth (1999) in Lieth et al (ed.) (1999) which are potentially of commercial value as cash crop or for other purposes.
From the number of species presented in that paper, we have chosen to discuss here first attempts to develop *Avicennia* species or varieties into vegetable crops.
The seeds of several *Avicennia* species have been shown to be edible. Foliage and seeds are used as animal fodder in several countries.
Several scientists within the EU CA "Sustainable Utilisation of Halophytes" have started to develop production systems, chemical analyses and irrigation techniques to grow *Avicennia* on large scale in desert areas in North Africa and Arabia.
Halophytes possess genes for salt tolerance. These may help to improve the salt tolerance of common crops. New molecular biochemical techniques were initiated to identify their position. As one of the model plants we took *Aster tripolium.*
Production plans, research design and first data from chemical and genetic analyses of this species will be discussed in this paper.

Keywords: Sustainable utilization, Halophytic cash crops, Saline production systems

Introduction

The increase of salinisation of soils is a general problem world wide. It is of special concern in countries with low rainfall and hot temperatures like Pakistan. Such countries have a high standard of irrigation practises where the farmers try to get the maximum efficiency from the irrigation water because this is the most costly production factor. The necessary drainage of some irrigation water leaves the farmer with a pond full of slightly saline water which he cannot use any more on conventional crops. The elevated evaporation from the irrigated field surface leaves often a residual salinity which reduces the production and quality of the following crop.

This condition has lead a group of European Scientists to cooperate in a concerted action "Sustainable Halophyte Utilisation in the Mediterranean and Subtropical Dry Regions" to promote the sustainable utilisation of halophytes. From the many potential applications of halophyte utilisation listed in Table 1 we concern ourselves in this paper with the saline production systems for food and feed, and this with salinities of irrigation waters up to seawater salinity and higher.

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Table 1. Utilisations of halophytes already existing and utilisation purposes that are investigated. From Lieth and Lohmann (2000)

1 Food	2 Feed	3 Wood	4 Chemicals	5 Landscaping	6 Ornamental
Starch	Starch	Fire	Industrial chemicals	Roadside	Potting plants
Protein	Protein	Building	Pharmaceuticals	Housing areas	Gardening
Fat	Minerals	Crates	Plastics	Dune fixations	
Vitamines					
7 CO_2-sequestration	8 Tertiary treatment	9 Industrial raw material	10 Unconventional irrigation water	11 Environmental protection	12 Wildlife support
Greenification	Water	Fiber		Coastlines	Species diversity
	Soil	Biomass		Turf	

Pakistan has in the Indus river delta several farming/fishing communities which use natural mangroves and other seawater receiving halophytic communities for the production of fodder and in some Pakistani farming communities are, therefore, a good group of practitioners with which new saline production systems based on cashcrop halophytes may be demonstrated to the needed third world countries all around the world in the dry, low latitude regions.

In addition to being potential cashcrops themselves, halophytes have the potential to improve common agricultural crops. Scientists try presently to raise the salinity tolerance of common crops with genetic manipulation. Little progress is made so far. A group among the EU concerted action scientists has started to identify some of the genes in halophytes which seem to enable these plants to tolerate higher salinities. If one compares the present successes of the genetisist searching for salt tolerant genes in glycophytes the level of salinity tolerance we find in halophytes is in a different order of magnitude. It might indeed be more efficient to try to insert the production and photosynthates allocating genes into feasible halophytes than trying to get higher salinity controlling genes into glycophytes. Both working hypotheses need to be followed. It is very clear that the increasing hungry population is faced with a drastic increase of salinity in the environment which reduces the productivity of our staple food crops drastically and that we need halophytes immediately to utilise light saline soil and unconventional irrigation waters.

If there is any vision for another green revolution in the near future it will be based on cashcrop halophytes either selected from naturally occurring halophytes or from halophytes or from traditional crops genetically altered for high salinity tolerance and the accompanying other changes in the production environment. Out of the many species of halophytes to be used in dry countries in the low latitudes we recommend *Avicennia* species for which we gained first successes in North African countries and the Arabian Peninsula.

From the *Avicennia* species is *Avicennia marina* native to Pakistan. It is highly productive at the coastline and with little further development this species could become together with other halophytes a great cashcrop for the farmers of Pakistan.

Avicennia germinans is a species native to the new world mangroves. We were able to transfer this species to Morocco where it grows in Agadir at several places under human protection.

This paper is divided into two sections:

1.- The development of saline irrigation production systems for *Avicennia marina* species and further cultivars.

2.- The initiation of genetic code assessment in halophytes on the model species *Aster tripolium.*

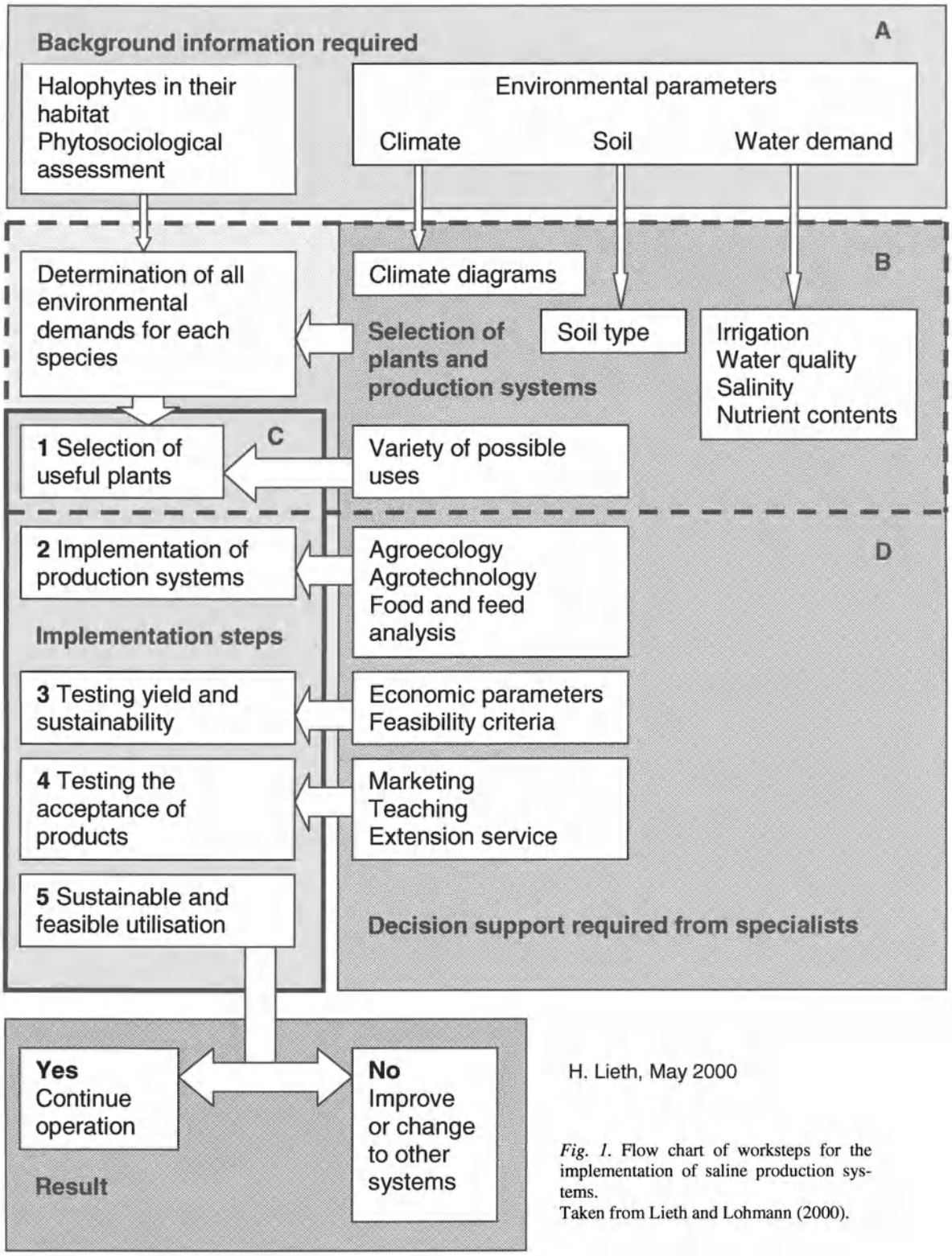

Fig. 1. Flow chart of worksteps for the implementation of saline production systems.
Taken from Lieth and Lohmann (2000).

Saline production systems

The development of saline production systems needs to follow the steps outlined in Figure 1.

In several pilot projects we have shown the ecological sustainability of such systems. They all require irrigation. Irrigation requires a large investment and operational cost. It is necessary, therefore, to harvest a marketable product with which the farming community can support itself. The number of parameters to be considered in the planning stage for saline production systems and these interdependencies are shown in Figure 2 taken from Güth (2000).

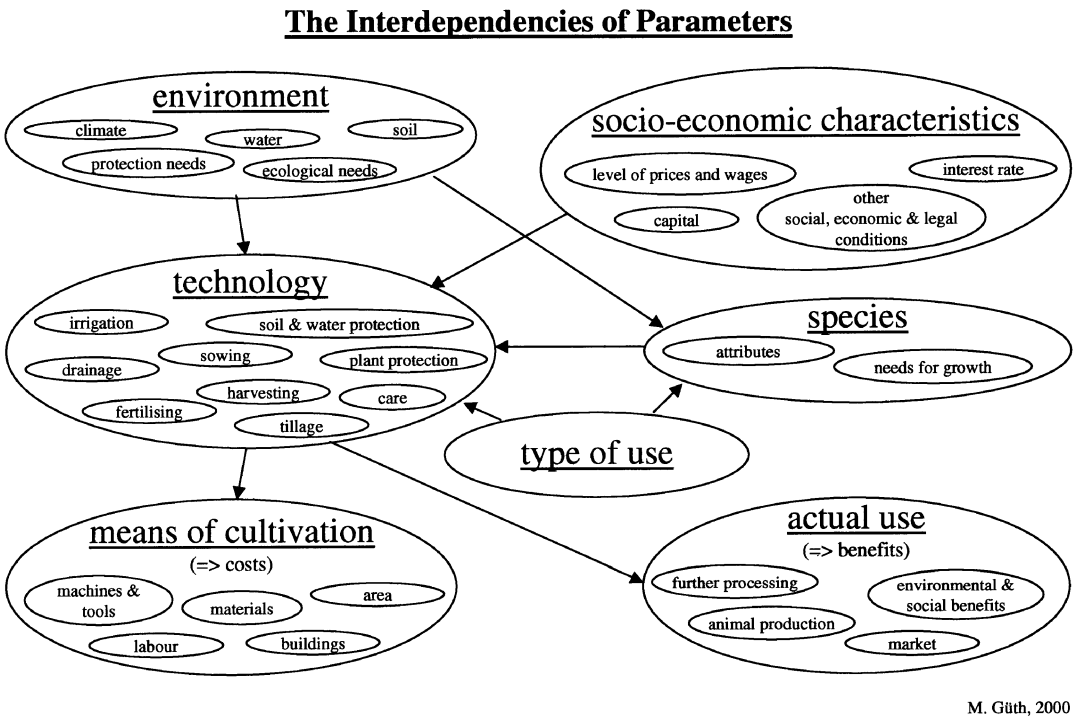

Fig. 2. Parameters to be considered for the development of cashcrops from halophytes after Güth (2001).

Following the rationale of Figures 1 and 2 we can discuss the requirements for cash cropping *Avicennia marina* (or any other similar halophyte).

Following the alphanumeric system of Figure 1 we can conclude for the background requirements in section A and B that the *Avicennia marina* population from the Indus delta is a potential cashcrop.

The climate of lower Pakistan will allow the production of this species with saline irrigation even in places where little growth is possible because of the lack of rain (see Figure 3).

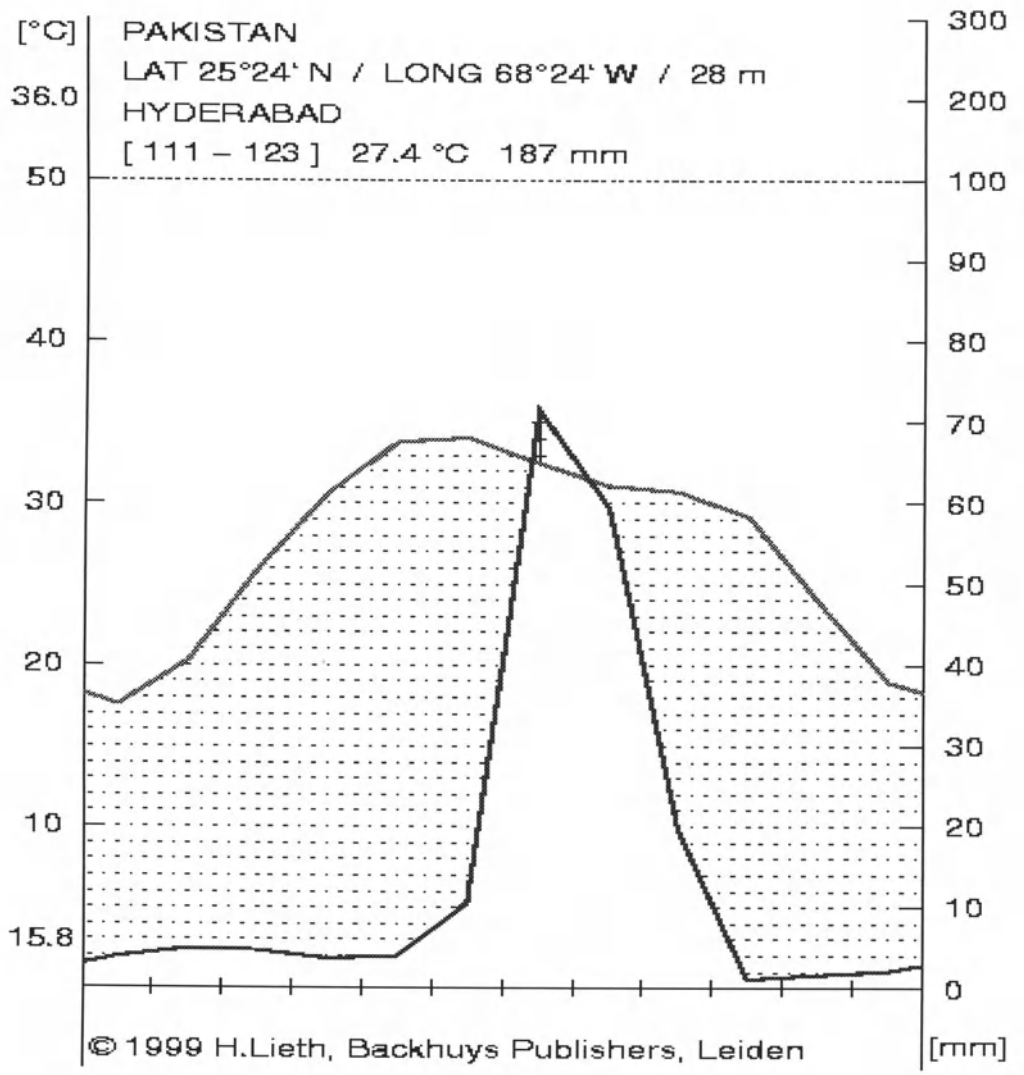

Fig. 3. Climate diagram from Hyderabad/Pakistan from the CD Rom published by H. Lieth et al (1998).

The soil in that region is only important with regard to the amount of irrigation water needed for the new cropping system. It might be noticed that the use of saline water for irrigation may alter the texture of the soil as was shown by Sardo in Sicily for sandy soil which was believed to be stable. For *Avicennia marina* is this only important if the systems falls dry.

Otherwise the plant grows perfectly in compact soils of any texture as long as the soil remains wet.

The water is best supplied by ditch or drip irrigation. The amount of water needed for each type of irrigation will let the farmer decide which irrigation type he will apply. We have shown that both cases are possible. Fertilisation is needed like in other crops. If seawater is used the addition of N and P is recommended. (Abdelly et al. (1999). Sleimi et al.(1999)).

Having all points of sections A and B covered we go to section C and check for step 1 the usefulness of *Avicennia marina.*

Avicennia marina can become a new general cashcrop for Pakistan. Because of its unreliable and low rainfall has Pakistan in wide regions difficulties in producing enough grain crops. The difficulties are still enhanced by the soil conditions. In many alluvial habitats gets the soil waterlogged, a situation which prevents common upland grain crops to be cultivated. The soil in such areas is often too salty for common crops as well. Several halophytic species have been tried, therefore, to fill the production gap (Aslam et al. (1999)).

Among the many species tried was one species missing, *Avicennia marina* which is abundantly growing will in the Indus delta region and is used there as animal fodder, for fuel, wood and as vegetable as well. We have shown that this species can be grown with saline irrigation in the desert of UAE like several other mangrove species. Since we have shown also that the fruits of this species are edible we can recommend this species to be planted further inland by using saline irrigation up to the salinity levels of seawater. The species produces naturally a large amount of fruits with a good quality for human consumption, but traditional breeding techniques could improve quantity and quality of production.

Avicennia marina can be grown in hedges like tea or similar perennial crops. Since the production technique is not yet known to the common farmer it seems necessary that demonstration plantations are developed in agricultural research stations as it is done for other halophytic crops in Dubai. With the large experiences the coastal farmers have with the utilisation of *Avicennia* it should be possible to develop a successful production system for Pakistan and India and get it accepted by involving the participatory Saline Agriculture Network Pakistan.

While the production and utilisation of *Avicennia marina* seeds to be achieved within a short time, the species may also be useful as donor for salinity tolerance genes as we will show in the next paragraph.

Section 2.Exploitation of the genetic potential of the halophytes *Aster tripolium* and *Avicennia marina* for the development of cash crop plants

Abstract. Basic characterization of salt stress related compounds from halophytes and the analysis of expressed genes together mediate insights into genetic control and response of stress and salinity tolerance which allows a good prediction on processes such as germination requirements for halophytes and cash crops under higher saline environment. In addition to learn more about the genes involved in physiological processes our aims are to use halophytes as donors of genes involved in salt tolerance, to establish marker genes for salt tolerance and to select suitable genotypes for the evolution of cash crop plants from wild-type halophytes.

I. Initial requirements.

At the beginning of the genetic characterization of organisms and the isolation of interesting genes a so called cDNA library need to be prepared. To obtain a cDNA library all mRNA molecules of an organism, in our case from *Aster tripolium*, are carefully extracted and transcribed in a reverse manner into DNA complementary to the RNA (cDNA). The cDNA synthesized represents all or at least most of the expressed RNA molecules in a cell and contains only the coding regions for the respective proteins without any introns. The cDNA is cloned into certain vectors which can be amplified in phages, bacteria or yeast. Now the cDNA can be screened with different methods for known salt tolerance genes, for marker genes or for cDNAs coding for unknown proteins which might be involved in salt tolerance.

II. Strategies for identifying genes which might enhance salt tolerance (*estg*, enhance salt tolerance genes).

The salt tolerant plants *Aster tripolium* and *Avicennia marina* are the molecular targets we have chosen for establishing a screening assay to identify genes which enhance salt tolerance (*estg*). We have chosen this species because it is used as cashcrop in many countries of Europe widely studied within the EU concerted action and has been tried as a catch crop in Pakistan as well. Plants were cultivated under low and high salt conditions followed by the subsequent isolation of their active RNAs.

Strategy 1:

The respective cDNAs were cloned into vector systems which allow the expression of the genes and therefore a screening on the protein level. The screening of the plant cDNAs expression libraries under different environmental conditions will be performed through functional complementation in bacteria and yeast to identify genes which might be responsible for enhancing plant salt tolerance and for genes which are essential in recognition of salt stress (Figure 4).

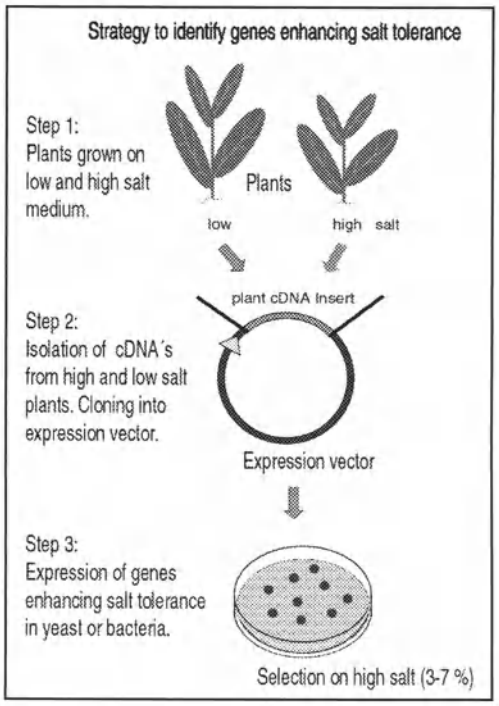

Fig. 4. A schematic presentation of the strategy for identification of estg. The vector system used in the cloning step depends on the expression system (bacteria, yeast or plant cells) which is used for the screening assay.

Strategy 2:

The respective cDNAs are cloned in certain vectors to carry out a substractive cDNA screening. All cDNAs expressed under low salt levels are substracted from/hybridised with cDNAs also expressed under high salt levels. The rest of the cDNAs might encode proteins which are involved in tolerance mechanisms protecting against high salt concentration. The Microarray technique, a modern high throughput method, is also very promising tool and might be a standard method in the very near

future for these kind of projects (Cushman and Bohnert, 2000).

Strategy 3:

To obtain information about metabolic processes which might be changed during adaptation to higher salt levels and enhance salt tolerance the expression of genes known from other organisms will be followed. The RNA abundance and therefore expression levels might differ in plants grown at low or high salt concentrations in the watering solution. These genes might be used as marker genes, for example for the selection of suitable ecotypes for further detailed analysis.

Strategy 4:

The protein expression level can also be analysed on 2-DE gel, which allows the reproducible separation of more than 2000 proteins in a single 2-DE gel. Changes in environmental conditions can now be directly compared on the expression pattern in a 2-DE gel. Subsequently a systematic analysis of those proteins (proteome) by a modern high throughput technique, the mass spectrometry (MALDI-TOF) enables the identification of expressed polypeptides (full length!) by accurately measurements of the masses of a protein (mixture). The identification of the correct protein sequence comes from alignments with peptides in nucleotide and protein databases with measured peptide masses. A requisite for the successful access is a certain preparation technique of membrane vesicles, organelles and protein complexes. Standard preparations will be obtained in future from *Aster tripolium* and *Avicennia marina.*

III. Current state of the experiments.

As an example of strategy we would like to introduce you into the current state of our experiments. It was shown that sulfolipid contents are increased in plants when treated with increasing NaCl concentrations (Stuiver et al., 1981). Therefore we hypothesize an increased expression of genes involved in sulfolipid biosynthesis in plants while treated with increasing NaCl concentrations. *Aster tripolium* plants were watered with tapwater (0%) and tapwater+3% NaCl for several days (Figure 5). Leaf material was harvested from the treated plants frozen in liquid nitrogen and mortared to a fine powder. RNA was extracted according to Chomszinsky and Sacchi (1987). About 15 µg total RNA was separated on a denaturing 1% agarose-formaldehyde gel (Figure 6, right). The RNA was transcribed into cDNA (Figure 6, left) and cloned into an expression vector (Figure 4). For a partial amplification of a gene involved in sulfolipid biosynthesis specific primers were constructed using highly conserved regions of the known gene *(sqdl)* from *Arabidopsis thaliana* and *Oryza sativa* (Benning, 1998). These primers and the synthesized cDNA (1 µg) as templates were used in a Polymerase chain reactions (PCR). A PCR fragment of the calculated size of about 700 bp could be amplified (Figure 7). PCR analysis indicates that *sqdl* is present in the cDNA library prepared from plants treated with 3% NaCl. However, this amplification product could only be demonstrated in PCR reactions with cDNA from the *Aster* plants watered with 3% NaCl as a template. The abundance of the *sqdl* gene was used to study cDNA libraries from halophytes for genes which are involved in salt stress. To our knowledge these are the first cDNA libraries from *Aster tripolium* and they are the basic tools for further analysis of halophytes on the genetic level as described above. Differential signals after salt treatment can now be selected on genomic and proteomic level for identification of estg.

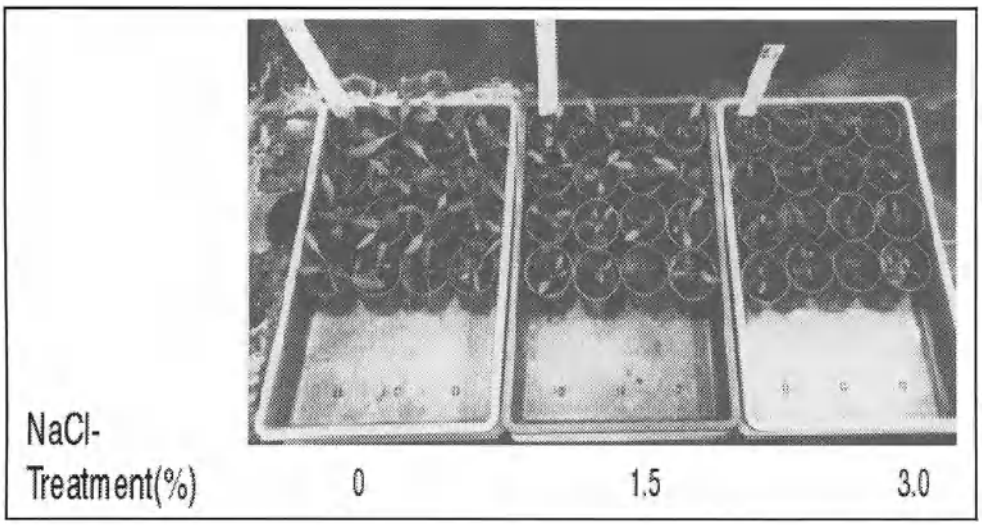

Fig. 5. Salt effect on growth of *Aster tripolium*, population Dollart. *Aster tripolium* plants shown are about 10 weeks old. From the left to the right: tap water, tap water plus 1.5% NaCl, tap water plus 3.0% NaCl. The photograph was taken about two weeks after beginning of the treatment.

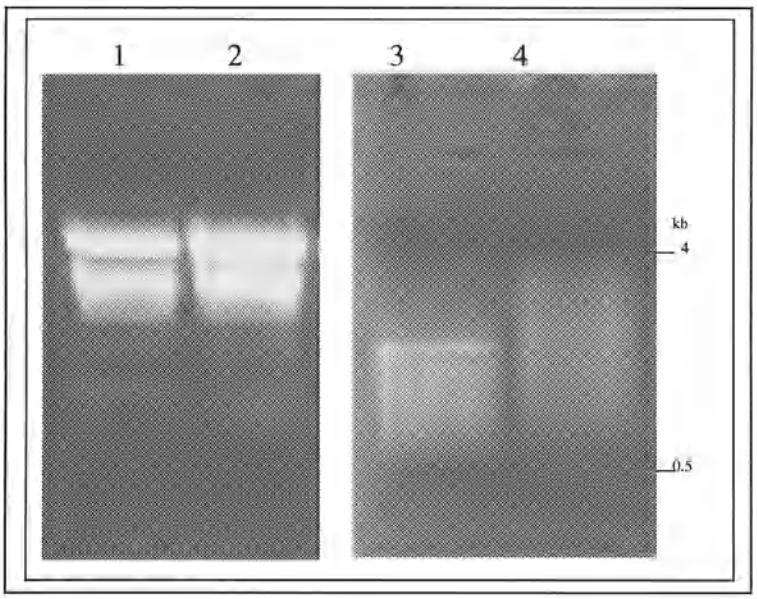

Fig. 6. RNA and cDNA-synthesis from leaves of *Aster tripolium*. RNA was extracted according to Chomszinsky and Sacchi (1987) from plants which were cultivated without additional NaCl or in presence of 3% NaCl in the tap water. About 15 µg total RNA was separated on a denaturing 1% agarose-formaldehyde gel (*lane 1*: 0% NaCl; *lane 2*: 3.0% NaCl). RNA obtained from *Aster tripolium* as shown here was used for subsequent cDNA synthesis. The corresponding cDNA is shown on the right (*lane 3*: 0% NaCl, *lane 4*: 3% NaCl).

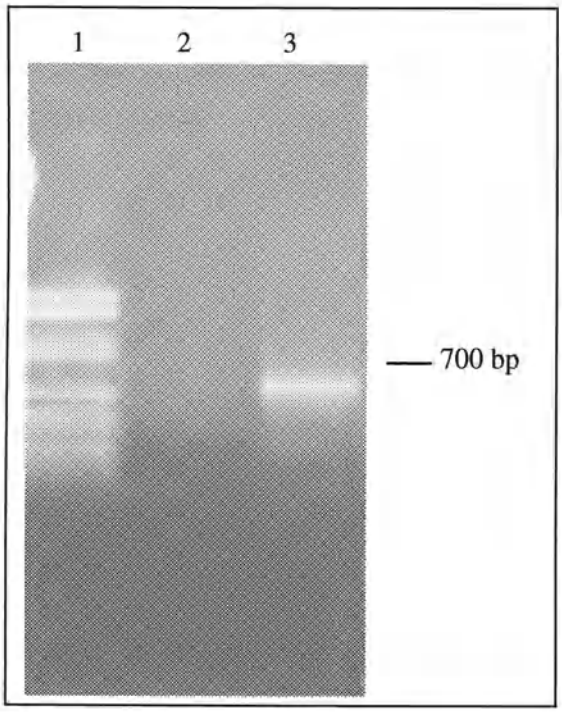

Fig. 7. sqd1 gene as an internal marker for salt stress. PCR on RNA extracted from NaCl-treated or untreated plants is analyzed on 1% ethidium bromide stained agarose gel. *Lane 1*: DNA-marker using *Hinf* I and *Bgl* I digested pBR328. PCR reaction on RNA obtained from NaCl-untreated plants (*lane 2*) or 3% (*lane 3*).

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