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THE INDUSTRIAL UTILIZATION OF MEDICINAL PLANTS WITHIN DEVELOPING COUNTRIES

Transfer of Technology for the Genetic Improvement*

Background paper

Prepared by

UNIDO Secretariat

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Preface

The Second Consultation on the Pharmaceutical Industry held in Budapest, Hungary, 21-25 November 1983, discussed the issue of development of drugs based on medicinal plants. A recommendation was made at the Consultation meeting to UNIDO to outline steps to be taken for future programmes with regard to transfer of technology for genetic improvement of medicinal plants.

This document has been prepared in pursuance of the above referred recommendation of the Consultation. 1/

1/ Contribution from Prof. S. Balasubramaniam, University Peradeniya, Sri Lanka, Prof. R. Gupta, Indian Council of Agricultural Research, Prof. Tetenyi, Inst. Med. Plant Research, Budapest, Hungary and Prof. V.H. Heywood, University of Reading in the preparation of this paper is acknowledged.

1. INTRODUCTION

Many plant species are used for the production of pharmaceuticals, perfumes, flavouring agents and beverages. The raw materials for these industrial processes are often procured from developing countries and processed into drugs and other finished products at centres located in developed countries. Some of the requirements of the pharmaceutical industy are obtained from species cultivated in well managed plantations or small holdings. Large proportions of these raw materials are, however, gathered from the spontaneous flora of different areas. The genotypes presently cultivated, their post-harvest handling, packing and shipping to centres of processing, determine the quality of the raw materials and the prices they fetch in international markets. Developing countries receive relatively low revenue from the export of these raw materials but they often pay high prices for drugs and other finished products they import for the health care of their people.

The plant based medicinal raw materials account for an appreciable percentage of the total plant-derived raw materials in world trade; an equal volume of trade (in monetary value) accounts for raw materials, utilized in perfumes, cosmetics, food flavours, deodorants and allied industries. There is an increasing trend towards plant based raw materials in preference to synthetic ones which provides sufficient evidence for the continuing increase in the volume of trade in raw materials. It is common knowledge that most of these plant species and their wild relatives are found in the spontaneous flora of the developing countries of Asia, Africa, and America and in the Mediterranean basin. Despite the continuous exploitation of wild growing populations for commercial purposes, these centres of primary origin (of important medicinal and aromatic species of plants) still possess a rich diversity of genetic materials of each species; these in fact are the building blocks for genetic improvement in terms of upgrading yields and enhancing desired quality traits under cultivation. This array of wild, growing, evolving gene pools of medicinal plants is a common heritage of mankind to provide for the health care needs of populations of all nationalities. The ecogeographic survey, description and sampling of the populations of the species and genepools involved, and, where appropriate, the preservation of seed or other samples in genebanks is a prerequisite for genetic improvement work in medicinal plants. This, by its very nature, of utility in a vital sector like pharmaceuticals, should receive priority of resources and efforts at international level. At its 4th World Plant Genetics Conference FAO indicated that the subject of medicinal plants was not within their area of priority interests. Accordingly, UNIDO has a role to play in coordinating all efforts directed towards the industrial utilization of medicinal plants, for the future welfare of mankind.

In this context, a programme for the genetic improvement of medicinal plants as reflected in the recommendation of the Second Consultation on the Pharmaceutical Industry assumes much importance to developing countries.

Such a programme would include evolving varieties that would suit the requirements of varied agro-ecological conditions such as poor soil conditions, periodic flooding, etc. without a need to compromise on yields or quality of produce.

This paper would discuss some of the salient features of a programme aimed at developing species of medicinal plants to suit these conditions. It may be of interest to record that a number of developing countries and centrally planned economy countries have national Research Institutes for improvement and cultivation of medicinal plants, e.g. South Korea, India, China, Indonesia, Philippines, Kenya, USSR, Bulgaria, Hungary, Romania, etc. which provides evidence how important these crops are to their national economies. Their research efforts suffer from several limitations such as: the lack of agreement for a free flow of genetic material, introduction and transfer of seed of improved cultigens producing higher content or composition of active principles; all these in addition to poor infrastructural facilities, and shortcomings in personnel and monetary resources. A collaborative effort at international level is therefore necessary to vitalize these institutions, fix priorities and support the free flow of genetic stock or genetically improved cultigens to member countries. The proposed programme should thus benefit the countries producung raw materials and basic phytochemicals whereas user industries elsewhere may benefit by increased flow of uniformly high quality and produce obtained through cultivation of improved genotypes of these medicinal plants.

The technology for the genetic improvement of food plants used as food crops is well established, but this type has not been very widely used for the breeding of improved genotypes of medicinal plants. In many developing countries, climatic conditions are favourable for plant growth throughout the year and labour is readily available and not costly. If suitable varieties or hybrids of high value medicinal plants can be genetically developed for different agro-ecological regions, they can be cultivated as export orientated commodities. This would help to improve the economic standards of the people of developing countries. Besides producing better suited genotypes for different regions, the technology for processing the harvested plant materials into concentrates, extractives, fractions or freeze dried preparations of high quality could be transferred to set up extraction units at locations close to areas of cultivations. Carrying out such preliminary processing operations, finally where possible into basic chemicals within developing countries can considerably reduce freight charges, post-harvest deterioration and loss of yield of pharmaceutically active constituents. This will also ensure a better quality of starting material for the pharmaceutical industries and better guaranteed prices for the suppliers of raw materials. UNIDO's programmes for medicinal aromatic and spice plants should continue to take cognisance of such steps for the industrialization and economic development, and above all, the strengthening of technological knowhow of many developing countries.

The United Nations Industrial Development Organization's Second Consultation on the Pharmaceutical Industry (Budapest, Hungary 21-25 November 1983) through its recommendations emphasized (a) the need for the compilation of a data base and a directory of plants used as therapeutic agents and (b) the steps to be taken for future programmes of genetic improvement of medicinal plants and their processing. This paper outlines some of the steps that would need to be taken by UNIDO and other UN agencies in collaboration with international research institutes and national agencies for the transfer of technology on the selection and genetic improvement of medicinal plants. The plan of work would be limited to: developing new and newer genetically upgraded cultigens for selected sets of agro-ecological conditions, consistent with the changing quality parameters of the user industries and making nucleus breeders' seed available to all those who would need such for commercial cultivation. The technology regarding final processing of medicinal plants into pharmaceutical preparations is outside the scope of this paper.

The genetic improvement of medicinal plants needs the active collaboration of economic botanists, breeders, agronomists, phytochemists and pharmacologists together with farm management and extensive service personnel. The methodology for breeding better varieties of crop plants is well established and there are many centres with expert staff, limited germ plasm collections, technical and other facilities. The techniques of classical breeding are well documented and widely practiced by agricultural and horticultural scientists. Mutation breeding too has been very successfully used for improving crops and farm animals. During the last two decades protoplast-fusion techniques and recombinant DNA transfer techniques have been attempted in order to modify the genetic makeup of microbes and plants. These approaches appear to be promising for the genetic manipulation of plants. The technology is available but it needs to be extended and adapted for the breeding of high yielding and improved quality medicinal plants.

In the process of embarking on genetic improvement of a selected list of priority plants, there is an urgent need to conserve the broad spectrum of the available genepools of these plant species, through nature reserves and establishment of arboreta, germ plasm collections, etc. and strictly maintaining the genetic purity of each collection under cultivation at national level. But the following steps would be needed to support a genetic upgrading programme:

(a) Preparation of three priority lists of 12 to 15 each with different levels of priority of important medicinal plant species based on volume of trade, value and users. Such a priority listing could be included in the plan of work which would benefit current large scale cultivations and processing. These lists would vary region or countrywise, and may be recorded as 1st priority list, 2nd priority list, 3rd priority list.

(b) Collection, evaluation, cataloguing, documentation and preservation of the genetic diversity of the selected medicinal plants in suitably selected "Centres" by linking national efforts and organizing a network of genebanks as base collections and for longterm storage.

(c) Selection and breeding (tradition and mutations) of these high priority medicinal plants through a network of research centres in countries having facilities, by supporting them under a phased programme of activities.

(d) Modern techniques of *in vitro* culture be explored for creating genetic variability and using it for breeding and selection of progenes besides rapid multiplication and cloning of medicinal plants. This would include use of protoplast-fusion and recombinant DNA transfer techniques where feasible for improvement of medicinal plants of very high priority at one or two selected centres of work.

The paper also highlights various areas in the field which could be handled individually or collectively by national, international research organizations and United Nations agencies.

2. Choice of medicinal plant for genetic improvement

It is to be accepted that list of medicinal plants used in different national pharmacopoeias and traditional systems of medicine is large and comprehensive but most of these continue to have limited utilization/demand outside the country/region. For the purpose of genetic improvement, it is necessary to identify most important medicinal species recognized widely in accepted pharmacopoeias of the world and having sizable cultivations so that the volume of trade and monetary value is large to merit the efforts made. There is likely to be conflicting claims for fixation of the priority but many of these species like Cinchona, Pyrethrum, belladonna, opium poppy, Dioscorea, Henbane, Mints etc. to have universal acceptance to be included in the plan of work. It is therefore advisable to formulate several working groups of scientists drawn from major growing regions and user industries to decide the priority lists for the programme and distribute the work to different known centres of research in countries already having the basic infrastructure for this work, or in countries where such infrastructure could be built without disproportionate initial outlay.

2.1 Improvement of selected medicinal plant species for national and regional needs

Whereas the main programme should be aimed at covering genetic upgrading of selected and most widely grown/used medicinal plants having a bulk demand in pharmaceutical industries, the importance of related species used in specific countries for the same phytochemical other industrial product cannot be ignored. In practice, these sources of raw materials are accepted by trade and this in a way widens the raw material base of the industry and avoids competition for marketing. This can be illustrated by Berberis asiatica (B. karistala etc.) yielding berberine hydrochloride in India-Nepal whereas Coscinium fenestratum is used as a source in Sri Lanka. Similarly anethole is produced from Pimpinella anisum in Mediterranean Europe whereas Illicium verum is the source in China. This genetic improvement programme thus envisages support to such selected species through linking-up research efforts in a particular region where it has important economic bearing or accord support to the national effort in this field.

3. Collection, maintenance and long term preservation of the genetic diversity of medicinal plants

As already indicated many medicinal plants that are employed for industrial processing or used in the different traditional systems of medicine are still gathered from the spontaneous flora of different areas. Only a limited number are cultivated in moderated sized farms or herbal gardens. With rapid dwindling and alteration of many natural habitats several species are fast disappearing. There is an urgent need for appropriate international and national agencies to initiate programmes of collection and maintenance of germ plasm centres or banks of endangered species of medicinal plants. Agencies such as the International Union for the Conservation of Nature and Natural Resources (IUCN) and UNESCO sponsored Man and Biosphere (MAB) Committees in different countries are already compiling lists of endangered plant and animal species. There are, however, several countries where such programmes have not got off the ground. There is a need to initiate steps to forge links between these UN agencies and also with other appropriate national organizations in activating and supporting programmes of collection of the vast number of species of underutilized medicinal plants. The task may appear gigantic but it can be achieved by collaborating with national botanical gardens and forest research institutes of the major phytogeographic regions of the world. Some of the more prestigious botanical gardens and research institutes have staff, low temperature storage facilities and laboratories for tissue culture. Seed banks with special storage facilities are also available at the major agricultural and horticultural research stations in some developing countries. Many species could be induced to form callus tissues and batches of these can be subcultured and stored under refrigeration on solid culture media in small containers that do not take up much space. They can be periodically subcultured and checked for somatic and other abberant changes or deterioration.

The genetic diversity of important crop plants like wheat, rice, maize, soybean, sugar cane, potatoes, oil seeds etc. are maintained at international institutes and many national research centres. Even horticulturally popular plants like chrysanthemums, rhododendrons and tulips receive attention from state agencies and commercial growers in Japan, Holland and other countries. The agencies send special expeditions to remote parts to collect genetically related taxa for the production of new hybrids and cultivars to cope up with new demands and situations. While food plants and ornamental plants have attracted the attention of international agencies and commercial establishments, there has been very little effort or organized programmes for the collection and preservation of the genetic diversity of medicinal plants. This is an area of activity that merits the attention of UN agencies particularly UNIDO in view of their industrial potential as well as national and international organizations, and pharmaceutical companies.

4. Selection and conventional breeding of a priority list of medicinal plants

Once a list of medicinal plants for genetic improvement has been drawn, breeders can select genotypes from catalogues of genepool available at Research Institutes and genebanks for improvement studies leading to development of new varieties. The development of suitable genotypes for different agro-ecological regions will enable developing countries to systematically cultivate their requirements of appropriate pharmaceutically important plants. The development of new plant types and varieties would allow the cultivation of some high value medicinal plants outside their present restricted areas of production.

Combination of suitable genetic traits such as high yields, drought tolerance, disease resistance etc. are brought about by selection and development of pure lines and subsequent crossing to combine the desired traits. From appropriate crosses, progeny testing and further breeding stable genotypes are developed. At present level, several medicinal plants are regarded as low yielding as regards the biological yield or content. In the case of medicinal and aromatic plants, genetic selection coupled with faster analytical screening procedures such as HPLC, TLC and gas liquid chromatography (GLC) will be helpful in identifying high quality and high yielding single plant genotypes. In a large population pure lines are established from these selections and they are then crossed with suitable parental types to combine desirable genetic characters. These techniques are now well established but they have to be adopted for specific medicinal plants chosen for genetic improvement, large scale cultivation and industrial processing into improved cultigens.

Conventional breeding is time consuming and expensive. It may take several years for the breeder to develop desired genotypes in many species. However conventional breeding procedures have to be pursued alongside or in combination with newer procedures like mutation breeding and protoplast-fusion techniques.

5. Mutation and ploidy breeding

Physical (U.V., X-rays, gamma radiation) and chemical mutagens have been used in the genetic improvement of crop plants including Dioscorea spp., Mentha spp. Hyocyamus spp. and *Solarium laciniatum*. The techniques, information and concepts emerging from these studies are well documented and form the basis for applied genetical procedures. Different ploidy levels can be produced in plants with colchicine and other chemicals. Karyological studies of natural populations and groups of related plants have shown that changes in chromosome number and other features occur naturally and have contributed to speciation in plants besides making useful genetic/chemical changes in the plants make-up and its metabolic end products and could be exploited for improvement.

The Food and Agricultural Organization (FAO) and the International Atomic Energy Agency (IAEA) have been very active in the use of isotopes and radiations and this expertise will be very helpful for future programmes of genetic improvement of medicinal plants.

6. In vitro culture techniques and micropropagation of genetically improved medicinal plants

Excised plant organs and tissue explants can be cultured under aseptic conditions on nutrient media of known chemical composition. A tissue explant can be induced to produce a growing mass of undifferentiated meristematic cells called a callus. Callus tissue can be subcultured and multiplied under controlled conditions. Manipulation of the hormonal composition of the culture medium often results in shoot morphogenesis and initiation of roots. From the many embryo like units or plantlets can be raised and grown in green houses and then planted out in the field. This method can be adopted to produce large numbers of genetically uniform and virus free planting material of horticultural, agricultural and forestry species.

The tissue culture tool can also be utilized in creating genetic variability and use the chosen variants through selective breeding to transfer a desired trait in a cultigen. Similarly different ploidy level parents are used for conventional crossing and screening progeny population for desired traits.

The isolation of single cell clones with a high capacity for active synthesis of one or more pharmaceutically useful secondary metabolites and the scaling up of these cultures to chemostat or fermentation type units, promises to be a new approach for the production of biologically or physiologically active compounds (compare penicillin production).

The possible advantges which might acrue from the studies on "soma clonal variation" should receive due consideration.

7. Protoplast fusion and recombinant DNA transfer techniques for the genetic improvement of plants

Intact protoplasts can be isolated using cell wall dissolving enzymes (cellulase, driselase, rhozyme and pectinase). The isolated mesophyll protoplasts can be induced to fuse forming multinucleate bodies. Sodium nitrate, potassium dextran sulphate, high pH in a medium containing Ca^{2+}ions have been used to promote protoplast fusion. More recently, polyethylene glycol has been used to induce protoplast fusion. Following fusion, the protoplasts regenerate cell walls, undergo mitotic divisions giving rise to a mixed population of parental cells, homocaryotic fusion products and heterocaryotic fusion products. In the early phase, one of the major obstacles for the wide application of somatic hybridization technology for genetic improvement of plants was the difficulty of recognizing and separating hybrid protoplasts (heretokaryon). Flourescence activated cell sorting has been used to isolate heterokaryons after protoplast fusion. There are other techniques as well for recovering the fused protoplasts or heterocaryons. These are then cultured on special media. The cell cultures derived from heterocaryons are then transferred onto to a solid culture medium and exposed to light. Green colonies are formed and shoot morphogenesis is induced in the green calli using appropriate culture media. The shoots can be made to form roots on media lacking phytohormones. After root proliferation, the plantlets can be transferred in a green house and later planted out in the field. The above is an outline of the preparation of protoplasts, their fusion and the sorting of the fused protoplasts or heterocaryons. The heterocaryons can be cultured and made to regenerate into autotrophic plants.

In unrelated genera and species, genetic exchange of material via pollination and fertilization does not occur due to the operation of sexual incompatability mechanisms. These barriers can be partly circumvented by the protoplast fusion technique. This technique enables plant scientists to bring about interspecific and intergeneric crosses and to evolve new somatic hybrids. The protoplast fusion technique is relatively new but it shows considerable promise for genetic improvement of crop and medicinal plants. So far production of somatic hybrids have been limited to Solanaceae and some genera of the Apiaceae and Brassicaceae (Cruciferae).

Protoplast fusion techniques, plant tissue culture techniques and micropropagation are promising techniques for the genetic improvement and multiplication of medicinal and aromatic plants. The training of personnel skilled in these techniques and the setting up of laboratories and centres for carrying out these operations will require heavy investment. A network of centres for carrying out genetic improvement of medicinal and other economically important plants can be identified and strengthened by UN agencies on a regional basis.

The Zimmerman method of electrofusion is widely used and the recent use of amines as fusogens may also be mentioned.

Deoxyriubonucleic acid (DNA) is the genetic material of all eucaryotic organisms. DNA transfer was first successfully demonstrated with microbial systems. The transfer of DNA segments between microorganisms has been successfully carried out in many laboratories around the world. DNA segments or genes synthesized in vitro using enzymes can be tagged with gene markers. The gene or gene segment is then transferred to a bacterium by means of a plasmid or bacteriophage. Through this procedure a particular trait can be specifically transferred to a particular organisms. This technique is still in the research phase and it may be some years before the technology becomes readily available to plant breeders.

8. Points for consideration

For industrial processing and for better economic returns from medicinal plants a steady supply of high quality raw materials is desirable. Accordingly, in order to upgrade biological yield and chemical content leading towards commercial cultivation of productive species, it is recommended that a specialist working group should identify the most important species to be taken into consideration for genetic improvement. The pharmaceutical industry could assist UN agencies to take an active role in initiating work along this line. The most essential steps required to implement progress in this area are identified in the following:

(i) Drawing up of a list of regions of maximum genetic diversity of selected plants and their near-wild relatives. Collection of the genepool from these regions by linking-up national efforts through international funding.

(ii) Evaluation, cataloguing and documentation of the collected gene-pool and selected national research centres for each crop. Conservation of the genepool into base collection and long term storage at selected genebanks in countries where these are (or may be) cultivated commercially. Ensuring free flow of genetic material to member countries through international agreement as done for food crops and horticultural plants.

(iii) Drawing up a detailed plan of work for selected medicinal plants for genetic improvement by conventional and modern breeding techniques.

(iv) Identification of a network of research centres to carry out the programmes on different plants and linking them through an internal programme/coordinator.

(v) Facilitating the production of nucleus breeders seed for free flow of genetic stock and improved cultigens. Conducting training courses in genetic improvement of medicinal plants for scientists, technicians and farm managers engaged in research and cultivation of medicinal plants.

(vi) Publication of documents on available cultigens and the characters to allow flow of materials and information to user countries in the field of medicinal plants.

(vii) Establishing liaison with other international organizations such as IUCN, IBPCR, WHO etc. interested in aspects of this same scientific field.

(vii) Drawing the attention of national governments to the need for collection and preservation of the genetic diversity of medicinal plants in botanical gardens, forest research institutes and other centres.

9. Abbreviations, Glossary of Terms

DNA	-	Deoxyribonucleic acid
GLC	-	Gas Liquid Chromatography
HPLC	-	Hig Performance Liquid Chromatography
FAO	-	Food and Agricultural Organization
IAEA	-	International Atomic Energy Agency
IUCN	-	International Union for the Conservation of Nature and Natural Resources
TLC	-	Thin Layer Chromatography
UN	-	United Nations
UNIDO	-	United Nations Industrial Development Organization
WHO	-	World Health Organization