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TRANSFER OF TECHNOLOGY FOR THE GENETIC
IMPROVEMENT OF MEDICINAL PLANTS

Prepared by
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Transfer of Technology for the Genetic Improvement of Medicinal Plants

1. INTRODUCTION

Many plant species are used for the production of pharmaceutical compounds, perfumes, flavouring agents and beverages. The raw materials for these industrial processes are mostly 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 industries are obtained from species cultivated in well managed plantations or small holdings. Large proportions of the raw materials are, however, gathered from the spontaneous flora of different areas. The method of collection, 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 technology for the genetic improvement of crop plants is well established but it 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 should wherever possible be set up at locations close to areas of cultivations. Carrying out such preliminary processing operations in developing countries can considerably reduce freight charges, post-harvest deterioration and loss of yield of pharmaceutically active constituents. This will also ensure better quality of starting materials for the pharmaceutical industries and better guaranteed prices for the suppliers of raw materials. UNIDO's programmes for aromatic and spice plants also recommends such steps for the industrialization and economic development and above all 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 selection and genetic improvement of medicinal plants. The early phases of processing the raw materials into extracts, fractions or crude preparations is, however, closely tied up with post-harvest handling of medicinal plants. The detail 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 taxonomists, breeders, agronomists, phytochemists, pharmacologists and production engineers. The technology for breeding better crop plants is well established and there are many centres with expert staff, 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 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 high quality medicinal plants.

Besides embarking on specific programmes of genetic improvement of a selected list of priority plants, there is an urgent need to conserve the broad spectrum of plant species used as therapeutic agents by the different cultures and people of the world.

This paper outlines various steps with regard to the following:

(a) Compilation and updating of information on plants used as therapeutic agents

(b) Collection and preservation of the genetic diversity of medicinal plants (Biosphere Reserves (*ex situ* conservation) and establishment of Biosphere Reserves (*ex situ* conservation))

(c) Preparation of a priority list of pharmaceutically important species that could be genetically improved for large scale cultivation and processing

(d) Selection and breeding of these high priority medicinal plants through a network of research centres and phased programmes of activities

(e) The adoption of *in vitro* culture techniques for the rapid multiplication and cloning of medicinal plants

(f) To explore the suitability of protoplast-fusion and recombinant DNA transfer techniques where feasible for the genetic improvement of medicinal plants of very high priority

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. Compiling and updating information on medicinal plants

The number of plants used as raw materials for drug preparations of the different indigenous systems of medicines of Africa, Asia and Latin America is extremely large. Many countries have lists, descriptions and illustrated accounts of medicinal plants in their local languages. The botanical identification of many of the species is confusing or incorrect and the nomenclature is usually outdated. Over the past two decades a large volume of phytochemical data has been accumulated for many of the medicinal plants used for instance in China, Egypt, India, Mexico, Pakistan and other centres of old civilizations. Many of these studies have been carried out by reputed laboratories in both the developing and developed countries. Also centres like the National Institute of Health in the United States, the World Health Organization and other national laboratories in China, India, Japan, South Korea and other countries have carried out extensive screening programmes on plant extracts and fractions for anticancer, antifertility, hypotensive and other activities. Plants and plant preparations used in traditional medicines are usually referred to by their local names (ginseng, iscador, senna, vasica syrup, zoapatle etc.). In many instances different species or species combinations are used for the same prescription in large geographic areas like India. For example Berberis spp., are used in the Himalayan foot hills and northern India while Coscinium fenestratum (Gaertn.)Colebr., is used by native (ayurvedic) physicians in southern India and Sri Lanka. There are several other examples of plant substitution for the preparation of decotions, syrups and pills. Phytochemical and biological screening in some cases have shown that some of the substitutes contain the same major phytoconstituents or secondary metabolites.

There is now an urgent need to collate, systematize and update the available information on medicinal plants. It is important to have the identity of medicinal plants authenticated by botanists. The work needs to be done preferably by a team of scientists with training and research experience in taxonomy, phytochemistry and pharmacology. Since the volume of information is large and multidisciplinary in nature, it has to be handled by experts or centres with access to good library and computer facilities. Many universities and other research centres have excellent computer facilities for data storage and retrieval. International organizations such as UNIDO, FAO/IAEA etc. may assist in collaboration with appropriate authorities in the establishment of links with a network of centres in preparing a complete list of medicinal plants giving information on the botanical name, family, geographic distribution, relative abundance, phytochemistry, pharmacological and other biological effects, toxicity and therapeutic value. Such a list will help in identifying potentially useful plants for further research and drug development. Besides one or more comprehensive compilations of useful medicinal plants, the international organizations can as a first step initiate the preparation of a priority list of species of medicinal plants used industrially for the preparation of important pharmaceutical compounds. UNIDO already has lists of medicinal plants that can be recommended for cultivation in some developing countries. The new list can incorporate information on the genetic diversity (number of varieties, clones, geographic races etc.) of the high value medicinal plants that should be cultivated for industrial processing and drug manufacture. This activity or programme will also serve as a nucleus for initiating programmes of collection and preservation of the available germ plasm of pharmaceutically important plants.

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

As already indicated medicinal plants used in the different traditional systems of medicine are usually gathered from the spontaneous flora of different areas. Only a limited number are cultivated in moderate size 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 needs the assistance of UN agencies including UNIDO, national, 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 prepared, breeders can select genotypes for further improvement and development. The development of suitable genotypes for different agro-ecological regions will enable developing countries to grow most of their requirements of 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. There is at present a shortage of raw materials for extracting diosgenin. Suitable genotypes of commercially worthwhile *Dioscorea* spp., are not easily available for cultivation, outside the present restricted areas of production. Once a selection or a new cultivar has been identified and genetically improved, it can be rapidly multiplied by *in vitro* micropropagation methods.

Combinations of suitable genetic traits such as high yields, 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. Many medicinal plants are low yielding as regards the plant parts harvested and they usually contain very low levels of the pharmaceutically active ingredients (alkaloids, saponins etc.). In the case of medicinal and aromatic plants, genetic selection coupled with analytical screening procedures such as thin layer chromatography (TLC) and gas liquid chromatography (GLC) will be helpful in identifying high quality and high yielding genotypes. Pure lines are established from these selections and they are then crossed with suitable parental types to combine certain desirable genetic characters. As mentioned earlier, 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.

Conventional breeding is time consuming and expensive. It may take years for the breeder to develop suitable genotypes especially of perennial woody 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. Mutagens have also been used with Drosophila, fungi (Neurospora, Sodaria etc.) and other organisms to produce mutants for basic research. 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 ecotypic variations and speciation in plants.

The Food and Agricultural Organization (FAO) and the International Atomic Energy Agency (IAEA) have been very active in the use of isotopes and radiations for genetic improvement of crop plants. Their experience and expertise will be very helpful for future programmes of genetic improvement of medicinal plants. UNIDO, IAEA and FAO could jointly initiate collaborative programmes for the transfer of technology for the 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 is now widely adopted to produce large numbers of genetically uniform and virus free planting material of horticultural, agricultural and forestry species.

Tissue culture techniques besides being a tool for basic research in the area of developmental biology finds wide applications in horticulture and agriculture. Sterile hybrids formed from interspecific and intergeneric crosses can be rapidly multiplied by in vitro micropropagation techniques. Some medicinal plants have also been used for in vitro tissue culture studies.

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).

As outlined later, in vitro culture techniques are important for somatic hybridization of isolated protoplasts and the subsequent culture of fused heterocaryons.

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 (Cocking, 1960). 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 (Kao and Michayluk, 1974). 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 by Galbraith et al (1985). There are other techniques as well for recovering the fused protoplasts or heterocaryons. These are then cultured on special media (Kao, 1977). 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 (Caboche, M., 1980). The shoots can be made to form roots on Murashighe and Skoog's (1962) medium lacking phytohormones. After root proliferation, the plantlets can be transferred into "Jiffy mix" and grown 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 other 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.

To illustrate the transfer of gene segment from "donor to a recipient" species the following study is quoted:

Deoxyriubonucleic acid (DNA) is the genetic material of all eucaryotic organisms. Marjorie Matke and Antonius Matke (1985) have been studying the factors that activate the seed storage protein genes in maize. The seed storage protein genes are active only in the ripening grains of the maize cob but not in the living cells of roots or leaves of this species. For the purpose of studying why the seed storage protein genes are active only in the ripening grains, they isolated the seed storage protein gene and the genetic information surrounding it. It is believed that the genetic material in the vicinity of the seed storage protein gene is partly responsible for the organ specific activity of this gene. In their studies, they have been able to incorporate the seed storage protein gene segment into the genetic material (genomes) of sunflowers and tobacco plants. They found the maize seed (grain) storage protein gene to be active in undifferentiated sunflower callus tissue but it remains inactive in tobacco callus tissue. At present, the reason for this divergent behaviour is not fully understood but research is being actively pursued in this area of gene activity. It is remarkable that a gene from a monocot (maize) is still active when transferred into the genome of a dicot (sunflower). In the case of tobacco it is possible to regenerate the entire plant from individual cells into whose genome extraneous genetic information has been so incorporated.

DNA transfer was first successfully demonstrated with microbial systems. The transfer of DNA segments between microorganisms has been successfully carried 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 bacteriphage. 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. Recommendations

For industrial processing and for better economic returns a steady supply of high quality raw materials is desirable.

In order to assure this it is recommended that national agencies in developing countries should take necessary action to identify the most important species which could be taken into consideration for genetic improvement. UN agencies in collaboration with pharmaceutical industry could take an active role in initiating work along this line. The most essential steps required to implement progress in this area could be identified in the following:

(i) Formulating strategies and initiating programmes of work on the genetic improvement of a priority list of medicinal plants by conventional and modern breeding techniques

(ii) Preparing guidelines and identify focal points or a network of research centres to carry out the programmes

(iii) 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

(iv) Conducting training courses to transfer the technology for the genetic improvement of medicinal plants for scientists, technicians and farm managers etc. engaged in research and cultivation of medicinal plants.

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10. List of abbreviations

DNA	-	Deoxyribonucleic acid
FAO	-	Food and Agricultural Organization
IAEA	-	International Atomic Energy Authority
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
GLC	-	Gas Liquid Chromatography