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Introduction: Research Need

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I. INTRODUCTION

Knowledge of plants and knowledge of healing have been closely linked from the time of man's earliest social and cultural groupings. The medicine man was usually an accomplished botanist. Even in historical times, botany and medicine continued to be virtually one and the same disciplines until about 1500 A.D. when the two began to separate from their close association, to the advantage of both sciences.

It is well established that industrialization has many direct and indirect effects on the human population. Increased stress is the most evident though offset by an increased health awareness among the people and better medical facilities. Nevertheless increase in diseases (mostly in urban populations) such as coronary heart diseases, diabetes, hyperlipidemia, AIDS and Cancer cannot be denied. This statement can be validated by statistics for just one disease, say Cancer (Figs. 1.1, 1.2).

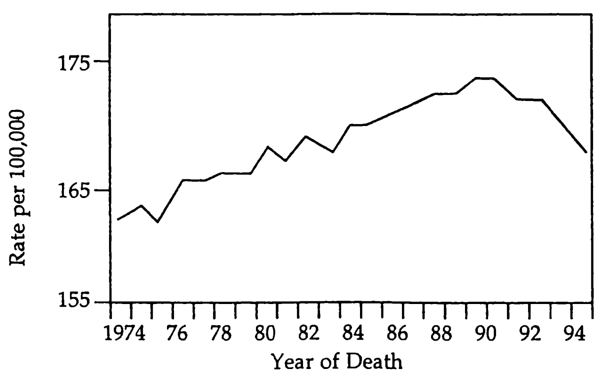

Fig. 1.1 Increase in deaths due to cancer

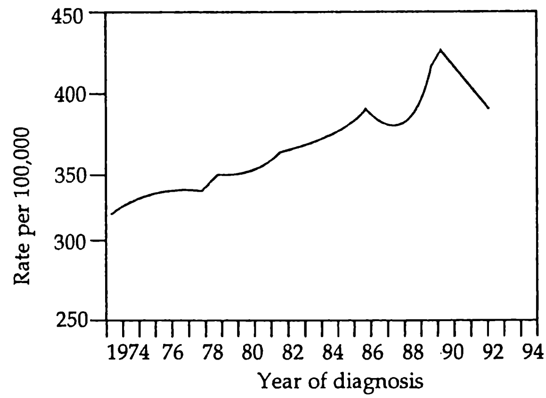

Fig. 1.2 Increase in incidence of cancer

Data on Cancer show both increased incidence of the disease as well as increase in per cent survival of patients due to better medical facilities. Although Cancer occurs in every country in the World, there are wide geographic variations in incidence. For males, incidence rates for all sites combined ranged from 493.8 per 100,000 in Australia and Tasmania to a low of 59.1 in Gambia. Rates for US males were about 350. This increase in the world population associated with increased health awareness and disease incidence will generate pressure on our natural resources, i.e., medicinal plants for drugs, cosmetics, health supplements, dyes and fragrances.

Besides medicines, mankind is almost completely dependent on plants for such requirements as food, shelter and clothing. However, most of the natural products are compounds biosynthetically derived from primary metabolites such as amino acids, carbohydrates and fatty acids and are generally categorized as secondary metabolites. These secondary metabolites are the major source of pharmaceuticals, food additives, fragrances and pesticides (Table 1.1). It is estimated that the market potential

Table 1.1 Plant-derived natural products and their industrial use

Industry	Plant product	Plant species	Industrial use
Pharmaceuticals	Codeine	*Papaver somniferum*	Antitussive
	Diosgenin	*Dioscorea deltoidea*	Antifertility agent
	Quinine	*Cinchona ledgeriana*	Antimalarial
	Digoxin	*Digitalis lanata*	Cardiotonic
	Ajmalicine	*Catharanthus roseus*	Antihypertensive
Agrochemicals	Pyrethrin	*Crysanthemum cinerariaefolium*	Insecticide
Food and drink	Quinine	*Cinchona ledgeriana*	Bittering agent
	Thaumatin	*Thaumatococcus danielli*	Non-nutritive
Cosmetics	Jasmine	*Jasminum* spp.	Perfume

for herbal drugs in the western world alone could range from US $4.9 billion to 47 billion by the year 2000. A similar situation exists for plant-based food additives, fragrances and biopesticides. Natural resources for plant-based chemicals are limited but the consumer preference for natural products is large.

II. MEDICINAL PLANTS EXPLORATION

Plants still remained, however, a great source of therapeutic agents until the beginning of the 20th century. With the development of chemistry in the last century, plants have been looked at as sources of new therapeutic agents. This investigation still continues and newer drugs of plant origin are being discovered every year. Some examples of drugs of plant origin discovered in the recent past are: muscle relaxants from South American arrow poisons; tumour-inhibiting agents from the family Apocynaceae, *Ochrosia, Podophyllum* and *Taxus*; tranquillizers from *Rauwolfia serpentina*, cortisone and sapogenins from several plants (e.g., *Digitalis, Dioscorea*); blood agglutins from the Leguminosae and phyllanthosides from *Phyllanthus*.

The importance of plant-derived drugs in modern medicine is usually not fully recognized (Table 1.2). Many chemical compounds of medicinal value are still obtained from plants for one or the other reason, such as Morphine, Codeine, Atropine, cocaine, caffeine, Quinine, quinidine, sparteine, ergotamine, vincaleucoblastine, taxol and a host of others. Then there are sundry drugs employed in crude botanical form, such as *Digitalis* leaf and *Rauwolfia* roots; or the extracts obtained from plants themselves, such as podophylline from *Podophyllum peltatum* and others. In fact, the complete Indian system of medicines, which is 3000 years old, is based on plants and their extracts.

Table 1.2 Important plant-derived natural products and their pharmacological activity

Medicinal agent	Activity	Plant source
Atropine	Anticholinergic	*Atropa belladonna* L.
Codeine	Antitussive	*Papaver somniferum* L.
Colchicine	Antigout	*Colchicum autumnale* L.
Digitoxin	Cardiovascular	*Digitalis purpurea* L.
Digoxin	Cardiotonic	*Digitalis lanata* L.
Hyoscyamine	Anticholinergic	*Hyoscyamus niger* L.
Morphine	Analgesic	*Papaver somniferum* L.
Pilocarpine	Cholinergic	*Pilocarpus* sp.
Quinine	Antimalarial	*Cinchona ledgeriana* Moens ex Trimen
Reserpine	Antihypertensive	*Rauwolfia serpentina* L.
Scopolamine	Anticholinergic	*Datura metel* L.
Steroids from Diosgenin	Antifertility agent	*Dioscorea deltoidea* Wall.
Vincristine, Vinblastine	Anticancer	*Catharanthus roseus* G. Don

Plants are not only the source of pharmaceuticals, they also provide chemicals of unknown and unusual chemical structures. These compounds provide new pharmacological properties and may serve as well as a starting material for more complex biologically active compounds. But we still don't know all the plants present on the planet Earth. Many more plants have yet to be evaluated chemically and pharmacologically.

A large number of plants have been screened in the last three decades for their chemical constituents as well as for pharmacologically active principles. These efforts have resulted in a plethora of compounds for further evaluation. Such programmes have been carried out independently by several institutes across the continents. Still another approach is to screen ancient literature on herbal medicine to select a plant. Unfortunately, the massive literature available is neither systematically combed nor critically examined. The National Cancer Institute, Maryland, USA is actively engaged in screening plants for their important pharmacological properties, and several hundred thousand plants have already been screened for their chemical and pharmacological attributes. With the advent of modern sophisticated tools of analytical chemistry, small samples can be evaluated, which has expedited the screening programmes.

Study of medicinal plants based on ancient literature and their investigation in the light of modern knowledge is known as Ethnology. However, the branch of medicine dealing with history, collection, selection, identification and preservation of crude drugs and raw materials is Pharmacognosy, while the branch dealing with the study of action of drugs is Pharmacology. Information about drugs and drug plants whose efficacy has been established, is available in various authentic books known as Pharmacopoeia and the drugs included therein are described as 'Officinal'.

III. RESOURCES: WILD AND CULTIVATED PLANTS

A large majority of these medicinal and aromatic plant raw materials are still collected in the wild. However, wherever the demand grew large from the organized sector of the industry and could not be met from natural resources, those species have been cultivated by introducing them into agriculture. Pharmaceutical and perfumery industries throughout the world have been making consistent endeavours to discover newer, more potent and cheaper sources of raw materials and their derived chemicals to broaden the product range in the trade. In recent years cheaper natural or synthetic sources of several phytochemicals have been developed. Cinchona and steroidal yam are examples of changing fortunes as plantation crops despite synthetics having captured world trade.

Cinchona bark and its principal alkaloid Quinine have been the main armoury of medical men to fight and control malaria in the last 200 years. However, the price of Quinine fell for the first time in 1951 to a low level of US $35 per kg but the discovery of the quinidine alkaloid in cinchona bark sustained its demand and supported the market price for some time; quinidine found use in cardiac medicine. This trend was maintained until the mid-70s because of resistant malarial strains for which synthetics did not work. However, it lost the fight to synthetics and the world demand for quinidine is now estimated around 110 t y^{-1} only.

The rise and fall of steroidal yam as a plantation crop has an equally fascinating story. In the early 70s, the steroids acquired a large market, exceeding a few billions, necessitating yam cultivation worldwide. Many developments in this field, such as production of these phytochemicals through chemical conversions in laboratories using cheaper starting chemicals, dampened its profitability as a plantation crop, however therefore, a large number of drug companies abandoned its cultivation. These examples clearly illustrate the volatile nature of raw materials trade in the agricultural sector of pharmaceuticals.

Plant cells are highly sophisticated chemical factories in which a large variety of chemical compounds are manufactured with great precision from simple raw materials. Plants are thus a very important renewable source of raw materials for the production of a variety of chemicals and drugs. India, being a tropical country and rich in biodiversity, produces and exports raw medicinal plants and their extracts. A number of plants are cultivated for this purpose (Table 1.3). These plants require biotechnological inputs to maintain their quality. According to one estimate, the present world trade in plant based dry raw materials and phytochemicals is around US $33,000 million.

Table 1.3 Area under cultivation of major medicinal and aromatic plants in India

Crop	Estimated Area (in hectare)	States where cultivated
Ashwagandha (*Withania somnifera*)	4000	MP, Raj.
Basil (*Ocimum basilicum*)	500	UP
Cinchona (*Cinchona officinalis*)	6-8000	Darjeeling (WB) and Ootacamund (TN)
Ipecac (*Cephaelis ipecacuanha*)	100	Darjeeling (WB)
Japanese mint (*Mentha arvensis*)	10,000	UP, Punjab
Jasmine (*Jasminum grandiflorum*)	2000	TN, AP Karnataka
Lemon grass (*Cymbopogon flexuosus*)	20,000	Kerala
Opium poppy (*Papaver somniferum*)	18,000	MP, Raj., UP (12 districts)
Palm Rosa oil grass (*Cymbopogon martinii*)	2000	UP, MP, TN, AP, Maharashtra

(*Contd.*)

Table 1.3 (*Contd.*)

Crop	Estimated Area (in hectare)	States where cultivated
Periwinkle (*Catharanthus roseus*)	3000	AP, Karnataka, Maharashtra
Psyllium (*Plantago ovata*)	50,000	N Gujarat, SW-Rajasthan
Rose geranium (*Pelargonium graveolens*)	2000	TN, Karnataka
Rose (*Rosa damascena*)	3000	Aligarh (UP), Udaipur and Ajmer (Raj.)
Saffron (*Crocus sativus*)	3000	Kashmir, MP
Senna (*Cassia* sp.)	10,000	Coastal Tamil Nadu
Vetiver (*Vetiveria zizanioides*)	Scattered in small areas	Kerala, TN, Raj., Karnataka

IV. ROLE OF BIOTECHNOLOGICAL APPROACHES

In-vitro grown plant cells and tissues have been used extensively for the production of secondary metabolites (Fig. 1.3). Depending on the objectives, a system such as callus, cell suspension or root/shoot culture is used. Once the *in-vitro* culture system is established it is used to achieve objectives through different approaches viz., micropropagation, genetic manipulation or cultivation in a Bioreactor (Fig. 1.4). The following applications of biotechnology are being pursued in the subject of secondary metabolites.

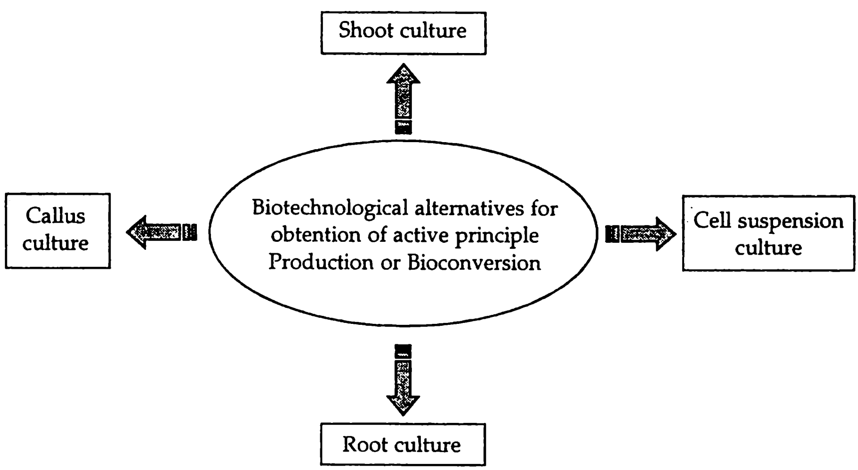

Fig. 1.3 Different ways to obtain a product *in-vitro*

1. Development of seed material for Domestication and micropropagation.
2. Continuous improvement of plants for secondary metabolites —through somaclonal and genetic variation.

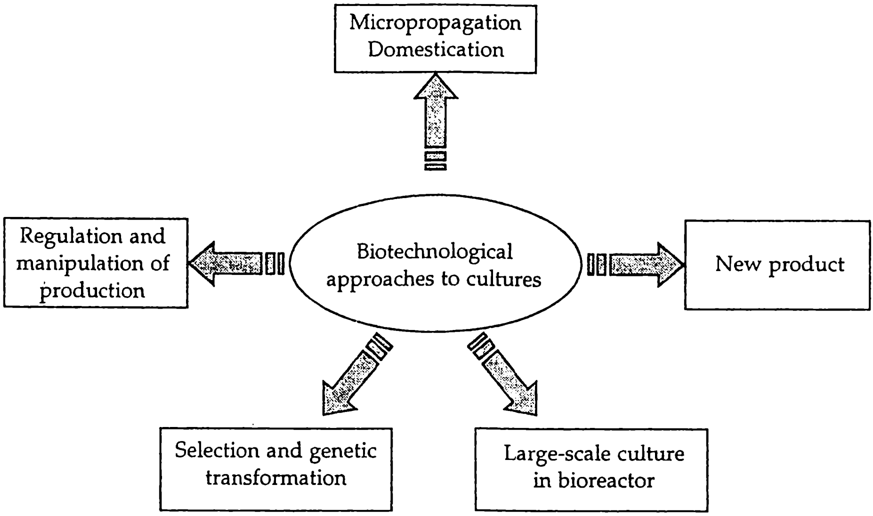

Fig. 1.4 Different approaches for the production of secondary metabolites

3. Understanding Metabolic pathways.
4. Alternative methods using Bioreactors (biomass); immobilization, organ culture (shoot/hairy root).
5. New compounds in tissue culture.

Development of Seed Material for Domestication

Plants are collected from their wild habitats for medicinal uses. This supply is unable to meet the demand if a plant finds multipurpose uses or applications. Under such conditions, uniform and large supplies can be assured only by systematic cultivation on a large scale. This requires planting materials such as seeds, tubers or other vegetative parts. There are several examples of insufficient availability of planting material necessitating, raising it through biotechnological method as the only way to get a sufficient amount. This approach has been used for the Domestication of such plants as *Digitalis* species, *Dioscorea* species, *Chlorophytum* species, *Commiphora wightii* and many others.

Micropropagation: Micropropagation is the most widely used application of plant tissue culture and micropropagation protocols for a large number of medicinal plants have been reported. Micropropagation is routinely used wherever a particular selected or unselected genotype has to multiply at an enormous rate to meet the demand of cultivators to replace the existing stock of material (perhaps having inferior characters such as low productivity, disease susceptibility, poor responsiveness) or for introduction to a new state/country for cultivation, e.g., *Atropa belladonna, Rauwolfia serpentina, Dioscorea floribunda, Mentha* species, etc.

Improvement—Somaclonal Variation and Genetic Engineering

Decline of potential for the production of active principle or essential oil content is a common feature with cultivation of medicinal plants and a continuous selection is required to maintain the high yield of active constituents for commercially viable programmes. Therefore, selection through conventional breeding as well as in cell culture is explored for obtaining high-yielding cultivars. Once selected, this material is multiplied by the micropropagation technique to provide seedling material to the farmers, e.g., *Mentha, Citronella* and *Cymbopogon* species. Though genetic engineering offers great promise for improving the existing genotype, the work is still in its infancy in relation to medicinal plants because more vigorous efforts are put into cereals and pulses. Genetic manipulation has been employed to obtain hairy root cultures of a large number of solanaceous plants and manipulation of biosynthetic pathways to obtain a desired product, e.g., change in flower colour by blocking the chalcone synthase gene responsible for flower colour.

Understanding Metabolic pathways

Plant tissue culture has been extensively used to understand the nutrition of the plant cell and the secondary metabolites it can produce. All the earlier work regarding the production of the active principle concentrated on the possibility of producing a compound by the cell culture of a plant species. Subsequent efforts were devoted to learning about the factors governing the production of secondary metabolites (optimization). Later, selection of cell lines for high yield of secondary metabolites and enzymes involved in the biosynthesis were investigated using radioactive precursors wherever considered necessary. Growth of the cell in a totally controlled environment of physical and chemical factors provides an excellent system for studying changes in the production of secondary metabolites, which are always present in small quantities. This basic information has provided significant clues for genes and their functioning, leading to genetic manipulation for biosynthetic pathways so as to obtain desired products by either blocking a pathway or enhancing the metabolic reaction.

New Compounds/Derivatives Production

Plant cell cultures have produced (a) new compounds previously not known in the intact plant, (b) new derivatives of a known compound and (3) new derivatives by biotransformation of molecules incorporated in the medium. Such results provide unlimited opportunity to screen the cultures for new compounds. It is presumed that production of new compound/derivatives might be due to altered gene function in the cultured cells compared to the *in-vivo* grown mother plant. Production of new alkaloids in cultures of *Catharanthus roseus, Ochrosia elliptica, Papaver somniferum* and *Ruta graveolens* are frequently cited examples.

Scaling up Technology Through Bioreactors

Plant cell cultures grown in a Bioreactor enable biomass production of a desired medicinal plant or its active principle through transformation. Such a system has been used for a long time for the production of antibiotics using fungi. The basic technology was derived from microbial systems and has been continuously improved for the growth of plant cells and organ cultures on a large scale. Bioreactors up to 20,000 litres have been designed and tested for the growth of plant cell cultures. Such a culture system produces a huge biomass in a short duration (10 to 20 days) but there are still several challenges to resolve before wider applications. However, several pharmaceuticals are produced or are on the verge of being produced at the commercial level using large Bioreactors, e.g., shikonin from *Lithospermum erythrorhizon*, taxol from *Taxus brevifolia* and berberine from *Coptis japonica*.

V. CONCLUSIONS

The geography of HIV infections shows that although sub-Saharan Africa still has by far the most infections (6 million adults), other regions are catching up fast. By the end of the millennium the number of people infected in Asia could rise to more than 3 million, most of them in India and Thailand. The situation is worse when we consider worldwide occurrence of various types of Cancers. With an increase in dreaded diseases associated with luxurious life style the demand for natural products will increase in the coming two decades. This demand will exert tremendous pressure on already scant natural resources. Therefore, biotechnological alternative methods hold great promise for meeting the demand and the challenge. The demand for natural products is also increasing day by day because of increased health and antipollution awareness as exemplified by the use of natural dyes instead of synthetic dyes for colouring clothes, biofertilizers and biopesticides. Health care products for improving skin colour and texture, antiageing and vitalizing drugs, medicines for hyperlipidemia and hypercholesterolemia are already in great demand.

VI. FURTHER READINGS

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