Medicinal Plants: Genetic and Pharmacological Perspective

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India is a known mega-biodiversity centre harbouring a multitude of medicinal plant species. Out of an estimated 17,000 higher plant species occurring in India, more than 1500 species are used over several centuries in the traditional systems of medicine viz. Ayurveda, Siddha, Unani and Amchi. The villagers and tribal folks spread across the length and breadth of the country make use of more than 7000 plant species through oral traditions (1). Nearly 3/4 of the herbal drugs and perfumery products used in the world are available in natural state in India. Therefore, the rich and varied species and genetic diversity of medicinal and aromatic plants, is one of India's important strengths and is the bedrock for all future bioindustrial developments.

Genetic diversity – a prerequisite for plant improvement

Nature has myriads of life forms on this planet among which variations are of ubiquitous occurrence. Genetic variations between individuals of the species were observed over the millennia and were considered as real 'hot spots' of evolution.

Genetic diversity is a fundamental component of biodiversity and represents all of the genetically determined differences that occur between individuals of a species in the expression of a particular trait or set of traits. Genetic diversity in natural populations contributes to long-term sustainability for several reasons as it increases the ability of populations to adapt to changing environments. The genetic variability is influenced by the geographical, seasonal and edaphic factors of the environment. The quality of adaptation of a population to different environmental conditions is directly dependent on its amount of genetic diversity. On the other hand, if a population lose genetic diversity they may experience fitness reductions and increased extinction risks due to inbreeding depression. Along with this, if individuals from other areas are allowed to inbreed with local flora, they reduce genetic diversity between populations and make them less fit for their survival in those particular conditions. In the wilderness of the tropics, plants grow in extreme situations along longitudinal, latitudinal and temperature gradients and therefore variations within and in between the populations of a species are not uncommon (2). A wide spectrum of simple and overlapping variations is now documented in plants (3, 4). In general, all observed variations are broadly grouped in to two categories: genetic and epigenetic.

Genetic variations in plants are strictly heritable i.e. truthfully passed on from one generation to another through seeds and do not change under conditions of cultivation. They occur invariably due to alteration in the genetic material and may affect both phenotypic and chemical characteristics of a medicinal plant.

Epigenetic variations, on the other hand, are mostly induced by the environment in which the plants grow and are also partially affected by the developmental events. Epigenetic changes in medicinal plants in general include morphological and chemical as well as physiological variations. Therefore, a great deal of information like morphological, biochemical, physiological and genetic is necessary before the observed pattern of variation may be interpreted. It is also true that beneath these intra-specific variations, there exists a fixed unchangeable genetic spectrum of characteristics that make up the species.

Genetic diversity – a fundamental resource for bioprospecting

Biodiversity is essential for bioprospecting to identify useful genotypes, genes and alleles of plant improvement. As Genetic diversity is the fundamental resource for bioprospecting, but it is rarely possible to predict which genes, species or ecosystems will become valuable for bioprospecting in future (5). Bioprospecting entails the research and exploration of biodiversity for commercially and economically valuable genetic and biochemical resources of medicinal and aromatic plants. Information regarding the genetic variations among medicinal plants are scanty although analysis of such variations holds great promise occurring to the location specific attributes of the herbs and the attendant diversity of plant-specific compounds of therapeutic and industrial value. It is also now understood that the loss of genetic variation within a given species (genetic depletion) is usually much more serious and occurs much earlier than the total extinction of the species itself. In the case of *Dioscorea zingiberensis*, plants collected during 1950s accounted a maximum of 17% diosgenin whereas during 1980s the content was reduced to 4% (6). Standing examples in this line are *Coleus forskohlli* (7), *Chamomilla recutita* (8), *Mentha arvensis* (9), *Aconitum napellu* ssp. Tauricum (10), *Valeriana officinalis* (11), *Mentha spicata* (12), etc.

The need for preservation of these genetic resources creates an incentive for determination of the genetic variability present within these plant species (13). Molecular markers have proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. Besides molecular markers, several other techniques are also available like isozyme, allozyme markers but they have their own limitations. A number of DNA based markers are now available for the effective quantification of genetic variation in plant species. RFLP (Restriction Fragment Length Polymorphism) and AFLP (Amplified Fragment Length Polymorphism) have been applied successfully and have provided considerable genetic information in a number of plant species. These techniques are slow and expensive and are not amenable for assessment of genetic variation in large scale population genetic studies. More recently, PCR-based RAPD and SSR (Simple Sequence Repeats) markers requiring small amount of DNA have also been developed. SSR markers have proved to be polymorphic but require nucleotide information for primer design. RAPD methodology overcomes this limitation; considerable polymorphic markers can be obtained with relative ease from minute amounts of genomic DNA without prior knowledge of sequence information (14). These RAPD markers are random and unlimited for number and are not affected by environmental factors (15). RAPD is convenient to operate, with good polymorphism can be used in analyzing genetic diversity and the relation between species has been used in analyzing the relationships between strains belonging to same genera and genetic diversity on many plants (16). Although RAPD is of dominant nature, several strategies have been put forward to minimize the dominance effects on genetic variation analysis (17, 18, 19). In occasional cases, RAPD is poor in reproducibility but this can usually be solved by optimization of reaction conditions (20). RAPD analysis provides information that can help define the distinctiveness of species and phylogenetic relationships at molecular level. Use of such techniques for germplasm characterization may facilitate the conservation and utilization of plant genetic resources, permitting the identification of unique genotypes or sources of genetically diverse genotypes. RAPD analysis has been used for genetic diversity assessment and for identifying germplasm in a number of plant species (21).

Ethnobotanical approaches to diseases – Indian perspective

In India, medicinal plants are widely used by all section of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in pharmaceutical preparations of modern medicines. According to National Health Experts, 2000 different plants are used for medicinal preparation for both internal and external use in India alone. Rigveda mentioned 67 plants having therapeutic effects, Yajurveda lists 81 plants and Atharveda 290 plants (22). Ayurveda, the Indian traditional system of medicine, is based on empirical knowledge of the observations and experience over millennia. More than 1200 diseases are mentioned in different classical Ayurvedic texts. A classical application of plant based medicines in the treatment of injuries is described in the Indian epic Ramayana, when Lord Rama's brother Lakshman lay mortally wounded on the battlefield in Lanka, medicinal plants from Himalayas were used for the treatment to restore Lakshman to fighting strength (23).

In Ayurvedic literature, there is a very wide-ranging conventional explanation of medicinal plants. There is a Shloka in Sanskrit, which says:

अयौग्यः पुरूषौ नास्ति, नास्ति द्रव्य मनौषम् ।
सर्वाणि सुलभाः यत, यौजकस्तत् दुलर्भम् ।।

The Shloka means, "There is no man on this earth who is incompetent and there is no plant which is of no medicinal value. In practice, a plant is called medicinal plant, when it is actually in use in some system of medicine" (24). The World Health Organization recently compiled an inventory of more than 20,000 species of medicinal plants.

With the realization that ethnomedicinal plants are a repository of numerous potential medicines, concerted efforts from India, Pakistan, China and other countries around the globe have been initiated to evaluate scientifically these plants for various biological and therapeutic properties as an alterative source of novel drugs. The traditional systems of medicine have now been recognized and accepted as alternative/complementary system of medicine for primary health care and as cure for some chronic diseases. This leads to the bioprospecting of medicinal plants. The search for valuable chemicals and genetic constituents of biodiversity is termed as bioprospecting.

Secondary metabolites – basis for the medicinal activities of plants

The beneficial medicinal effects of plant materials typically result from the combinations of the secondary products called as secondary metabolites present in them. Plant species still serves as a rich source of many novel biologically active compounds, as very few plant species have been thoroughly investigated for their medicinal properties. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (25). Important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. These groups of compounds show antimicrobial, antioxidant and anti-inflammatory effects and serves as plant defense mechanisms against pathogenic microorganisms, free radicals and inflammation.

Traditional physicians and village doctors collected herbs of certain morphological attributes (flowers, fruits and leaves) and preferred root drugs of specific color, smell, size, fibrous content, itching quality etc., obviously from locations known only to them. Even within a medicinal plant species, sometimes one variety was preferred over others. It is also not surprising that curative properties of a plant species change according to seasons or developmental stages and hence

Vaidyas prefer to collect required plants or their parts during certain periods only (26). This is because several factors may affect the levels of the active principles of plant material during collection, processing and storage. Indeed, biotic and abiotic stresses exert a considerable influence on the levels of secondary metabolites in plants (27). It is known that climatic conditions and water available in the soil can change the vegetal secondary metabolism and alter the composition of active constituents throughout the seasons of the year (28). For example, plant phenolics show marked qualitative and quantitative variation at different genetic levels (between and within species and clones) and between different physiological and developmental stages. They also vary in response to environmental factors, such as light intensity and nutrient availability (29). Thus, temporal (30) and geographic variations (31) in the phytochemicals are very common among plants.

Infectious diseases and plant antimicrobials

Infectious diseases account for approximately half of all death in tropical countries (32). Infectious disease results from the interplay between pathogens and the defense system of the hosts they infect. The appearance and severity of disease resulting from any pathogen depends upon the ability of that pathogen to damage the host as well as the ability of the host to resist the pathogen.

Medicinal plants have been used for centuries as remedies for infectious diseases because they contain components of therapeutic value (33, 34). In recent years, various secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents against various infections. Studies indicate that in some plants there are many substances such as peptides, tannins, alkaloids, essential oils, phenols, and flavonoids among others which could serve as sources for antimicrobial production. These substances or compounds have potentially significant therapeutic application against human pathogens including bacteria, fungi and viruses (35, 36).

The development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (37, 38). Antibiotic resistance has become a global concern (39) as the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug-resistant pathogens (40). Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for the development of novel drugs because of the great diversity in their chemical structure. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (41). Therefore, researchers are increasingly turning their attention to ethno-medicine, looking for new leads to develop more effective drugs against microbial infections (42) and this has led to the screening of several medicinal plants for potential antimicrobial activity (43). Scientists from divergent fields are investigating plants with an intention to discover valuable phytochemicals which have inhibitory effects on all types of microorganisms *in vitro* (25).

Free radicals and plant antioxidants

Free radicals, formed as a result of various metabolic reactions within the body and are the unstable species that react rapidly and destructively with biomolecules such as protein, lipid, DNA, RNA in the body. Uncontrolled generation of free radicals is associated with lipid and protein peroxidation, resulting in cell structural damage, tissue injury or gene mutation and ultimately led to the development of various health disorders such as Alzheimer’s disease, cancer, atherosclerosis, diabetes, hypertension and ageing (44). Antioxidants play an important role in defending the body against free radicals damage. Antioxidants refer to a group of compounds that are able to delay or inhibit the oxidation of lipids or other biomolecules and prevent or repair the damage of the body cells that is caused by oxygen (45). The beneficial effects of antioxidants on promoting health is believed to be achieved through several possible mechanisms, direct reaction with and quenching of free radicals, chelation of transition metals, reduction of peroxides and stimulation of the antioxidative enzyme defense system (46). Phenolic compounds such as flavonoids, phenolic acids, diterpenes and tannins have received attention for their high antioxidative activity (47). Recently, and mainly due to undesirable side effects such as toxicity and carcinogenicity of synthetic additives, interest has considerably increased for finding naturally occurring antioxidant compounds suitable for use in food and/or medicine. Plant phenolic compounds have attracted considerable attention for being the main sources of antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Flavonoids and other plant phenolics, such as phenolic acids, tannins, lignans and lignin, are especially common in leaves, flowering tissue and woody parts such as stem, bark and roots of plants (48). Many synthetic antioxidants such as BHA (Butylated Hydroxy Anisole), BHT (Butylated Hydroxy Toluene) are very effective but they possess certain health risks and toxic properties to human health. Therefore, the search for natural antioxidants of plant origin has gained momentum in recent years (49, 50).

Inflammation and plant anti-inflammatory agents

Inflammation, which is a pattern of response to injury, involves the accumulation of cells and exudates in irritated tissues that allows protection from further damage. Inflammatory cells produce a highly complicated mixture of growth and differentiation cytokines as well as biologically active arachidonate metabolites. In addition, they possess the ability to generate and release a spectrum of reactive oxygen species, reactive nitrogen species and free radicals during oxidative burst. The main anti-inflammatory agents are steroids such as prednisolone, prednisone, dexamethasone and betamethasone and non steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, naproxen, indomethacin, ibuprofen and flurbiprofen. The steroidal drugs are used as anti-rheumatoidal agents and are also beneficial in asthma to control inflammation. The NSAIDs are most widely used among all the therapeutic agents not only to treat acute inflammatory conditions but also for chronic diseases such as osteoarthritis and rheumatoid arthritis. The prolonged use of both steroidal and non-steroidal anti-inflammatory drugs is associated with various side effects such as peptic ulcer formation, disturbed renal function, suppression of the response to infection or injury, osteoporosis, muscle wasting and weakness, euphoria, cataracts, glaucoma and raised intracranial pressure (49). Currently much interest have been paid in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agents that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation responses are involved in amplifying the disease process.

Conclusion

Genetic diversity is a prerequisite for any plant improvement program and it is a fundamental source of bioprospecting. The medicinal properties of the plants are due to their secondary metabolites which are extensively affected by the genetic level changes that can be detected by several techniques like molecular markers. So there is an urgent need to correlate the genetic diversity of medicinal plants with the pharmacological behavior.

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