Importance of Using DNA Microarray in Studying Medicinal Plant

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Medicinal Plants:

Medicinal plants promised a positive concept of health based upon certain basic principles of physical, chemical and biological sciences in present busy life. Drugs from these are said to be potentially safe casting no side effects over synthetic drugs like antibiotics, analgesics, anthelminitics etc. Pyrostegia venusta (Ker Gawl.) Miers, Bignoniaceae, is native to the Brazilian Cerrado and popularly known as “cipó-de-são-joão”. Pyrostezia venusta have reported folklore used as medicinal plant for common diseases of disease of eye, nose, etc. The flowers of P. venusta are used as a general tonic and a treatment for diarrhea, vitiligo, cough, and common infections and inflammatory diseases of the respiratory system. The flower consist of gallo-tannic acid and glycoside bellericanin, which is bitter, pungent acid, digestible, laxative, anti-helminthic, anti-bronchitis, sore throat, anti-inflammatory, anti-asthamic. The fruit in combination with other drugs is prescribed for snake bites.

Economical importance:

Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, after a boom of allopathy these days herbs are staging a comeback and herbal ‘renaissance’ is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Over 70% of the world population depends mainly on plants and plant extracts for health care. Thus business world estimated that plant derived drugs may account for about Rs.2,00,000 crores. Presently, India contribution is less than Rs.200 crores which is growing steadily. The annual production of medicinal and aromatic plant’s raw material is worth about Rs.200 crores and it supposed to be raised to US $5 trillion by 2050. Even developed countries such as United States constitute 25% of the total drugs as plant drugs, while in most populated as well as fast developing countries such as China and India utilizing about 80% plant based drugs.

Epidemiology:

These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine. At present it is reported that there are more than 80,000 are medicinal higher plant species on earth. Among plant based drug producer, India is one important biodiversity centres with the recognition of over 45000 different plant species. India’s biodiversity have 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). This constitute, about 15000-20000 plants with good medicinal value. Of these 7000-7500 species are used for their folk medicinal values since ancient time.

Source of plant drugs:

Plant drugs can be derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. and even from excretory plant product such as gum, resins and latex. Presently, Allopathic medicine has also been derived from a number of plants which form an important segment of the modern pharmacopoeia. Few important chemical intermediates needed for manufacturing the allopathic drugs are also from plants origin (e.g. diosgenin, solasodine, b-ionone).

Importance of Medicinal Plants in Modern Age:

Traditional systems of medicine practice have several benefits on many accounts like population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases. About 80% of world population can not afford the products of the Western Pharmaceutical Industry and have dependence only on the use of traditional medicines which are mainly derived from plant material. In many of the developing
countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of major portion of world population although many under developed countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future.

**Need Of Scientific Analysis:**

Plants can synthesize variety of biochemical products because of their metabolism, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially used in a number of pharmaceutical compounds. However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost, and selection of the superior plant stock and over exploitation by pharmaceutical industry. Investigators have reported fractions of flowering plants that have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Presently, in market we can see many plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine. In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, the scientific studies of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants have its own importance. A major lacuna in ancient medicine preparation (like Ayurveda) is the lack of drug standardisation, information and quality control and can fail to give desired activity. This is now known that activity of the plants extract is the synergistic effect of its various components. In the absence of pharmacopoeia data on the various plant extracts, it is not possible to isolate or standardise the active contents having the desired effects.

**DNA -Micro Array:**

Worldwide, there are about 400 known families of flowering plants, of these 315 are available in India. World Health Organisation has also suggested around 21,000 plant species with potential medicinal values. To develop phytomedicines it would be essential to adopt a holistic interdisciplinary approach, based on scientific understanding of the plant systems, as well as new innovations and their conservation for utilisation in future on a sustainable basis. In our opinion these task can be achieved by using DNA chip technology. Recently, DNA micro-arrays have become an accepted and functional tool with which to meticulous study gene expression in cells and tissues in response to various agents and treatments. Theoretically, one can evaluate, all together, changes in gene expression (transcriptome, metabolome, kinome etc.) of the entire genome under single study. Therefore, micro-array tools have been applied by many investigators involved in cancer research, differentiation and development, toxicology, and the effects of pharmaceuticals on cells and animals. Additionally, few latest studies have shown the application of technique for revealing the novel response of important genes which were unstudied previously. Nevertheless, comparatively a small number of attempts have been made so far to evaluate herbal medicines, even though a number of relevant questions can be solve on applying this.

Already at a relatively early stage of its application in plant science, DNA microarrays are being utilized to examine a number of metabolic activities including the circadian clock, plant defence, environmental stress responses, fruit ripening, phytochrome A signalling, seed development and nitrate assimilation. Novel insights are obtained into the molecular mechanisms co-ordinating metabolic pathways, regulatory and signalling networks. Exciting new information will be gained in the years to come not only from genome-wide expression analyses on a few model plant species, but also from extensive studies of less thoroughly studied species on a more limited scale. The value of microarray technology to our understanding of living processes will depend both on the amount of data to be generated and on its intelligent analysis and application with other biological knowledge arising from functional genomics tools for delineation of genome, proteome, metabolome and phenome.

**Construction of DNA chip:**

Southern hybridisation technique was introduced by E M Southern in 1975. In this method, the gel with separated intact DNA fragments would get denatured in a basic solution and placed in a tray. A porous nylon or nitrocellulose membrane is subsequently laid over
the gel, and the whole weighted down. All the DNA restriction fragments in the gel transfer as single strands by capillary action to the membrane. All fragments retain the same pattern on the membrane as on the gel (Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by electrophoresis. J. Mol. Biol. 98:503-517.). Hybridisation is the foundation behind the technique of DNA microarrays. Hybridisation is the reaction that concerns with the fluorescent probes and the DNA on the microarray. The hybridisation conditions also depend on the application of the array like detection of mutations which requires high hybridisation stringencies occurs at lower salt concentration and higher temperature, for short time periods (hours). Using microarray for “expression monitoring” requires lower stringencies, to ensure overnight low-copy number sequences annealing at lower temperatures and higher salt concentrations. After this overnight hybridisation, the reaction can be scanned using a laser confocal scanning microscope, which read each spot of DNA and separately measures the fluorescence for each dye. This produces data to determine the ratio, and in turn the relative abundance of the sequences of each specific gene in the messenger RNA or DNA samples. The comparative study of hybridisation pattern can then be used to identify the genes that are expressed differently in the tissues or cells. The principle of the hybridisation reaction is to expose two single-stranded DNA sequences to each other, and measure the amount of double-strands that form. Microarray technology is a modern automation of southern hybridization technology combining miniaturization and the application of fluorescent dyes for labelling. The fluorescent dyes provide combination of two differently labeled samples in a single hybridization experiment with reduced experimental error. Miniaturization helps in developing relative expression levels of large numbers of genes in lesser time then traditional hybridization system with a high degree of sensitivity. There are two fundamentally different microarray-based technologies are available for large-scale expression analyses. First, photolithographic method in which high-density spatial synthesis of oligonucleotides, that contain up to a few hundred thousand distinct elements (Fodor et al., 1991). Construction of such arrays requires prior sequence knowledge as well as complicated design and production methodologies (Lipshultz et al., 1999). The second method utilized PCR-amplified cDNA clones and the resulting arrays are referred to as cDNA microarrays or printing cDNA microarrays. The cDNA microarrays contain pre-synthesized nucleic acids which are mechanically deposited, similar to ‘ink-jet’ printing onto a solid surface. That provides more flexible design for the fabrication of microarrays (Duggan et al., 1999).

**Photolithographic microarray production:**

Synthesis of oligonucleotides on a surface using photolithography is used to formulate high-density oligonucleotide microarrays. The most widely used method is the Affymetrix GeneChip technology. Photolithographic microarray fabrication involves DNA synthesis directly on the solid carrier surface by using combinatorial chemistry methodology. This ‘in situ’ fabrication technique was developed by Affymetrix, and is used to produce their GeneChips. They make use of semiconductor technologies. In it mercury lamp is used, and the light activates modified photospecific versions of the four DNA bases to solid surface, derivatized with chemical linkers containing photolabile protective groups with presence of photolithographic masks which ensure the DNA synthesis is stimulated in defined positions - the masks predetermine which of the nucleotides are activated. Subsequently, the surface is flooded with a modified nucleotide to be coupled to the activated region of the chip. A repeated series of steps involving selective activation of specific regions and nucleotide coupling allows parallel oligonucleotide synthesis at many locations (Lemieux et al., 1998).

Microarrays presently produced by Affymetrix normally contain 25-mer oligonucleotides within 20–24 ?m feature size. Typically, for expression monitoring, 16 oligonucleotide probe pairs (16 perfect match (PM) and 16 additional mismatch oligonucleotides (MM) for increased sensitivity and specificity of detection) are designed on non-conserved regions of a gene (http://www.affymetrix.com). The MM oligonucleotide is identical to the PM except for a single-base difference at the central position. A single microarray for expression analysis containing more the 400 000 features will therefore represent ca. 13 000 genes. Compared to the production of cDNA microarrays, the production of oligonucleotide arrays has the advantage that only sequence information and oligonucleotide design are pre-requisites, while handling of clones, primers, PCR products and so on is avoided. Oligonucleotide probes complementary to the known reference sequence (non-overlapping if possible) are usually selected to cover regions of 200 to 300 bases of the gene, cDNA or EST. Apart from the empirical composition parameters used for their design, other criteria such as uniqueness compared to family members and other genes, should also be
taken into account during probe selection.

**Printing CDNA microarrays production:**
cDNA microarrays are habitually made-up on solid surfaces generally microscope slides of glass. In order to boost the adhesion of probes as well as to lesser background and to restrict spreading of the droplets, the slides are pre-coated with substances like poly-lysine or amino silanes. There is at present two processes i.e. either contact printing or non contact printing. In contact printing method, either solid or split pins are dipped into the DNA solution then a micro-droplet subsequently deposited upon direct contact with the solid surface of the array. The system uses a motion control structure that spots or prints an accurate amount of sample probe onto multiple surfaces (often 50 to 100 microscope slides) in a sequential operation. According to objective, one can use contact printing typically to produces sub-nanolitre droplets at a pitch of 100–250 µm. Presently, mechanical spotting mentioned above is most common way for the fabrication of cDNA microarrays.

Non-contact printing consist a controlled ejection of small (nano- to picolitres) volumes of DNA solution from a dispenser onto the surface from a defined distance which allows flexibility in printing volume. Universally, non-contact dispensing uses various types of ink-jet technology (e.g. thermal, solenoid, piezoelectric) for droplet generation and delivery (Okamoto et al., 2000).

cDNA microarrays produced by ink jet printing may contain thousands of array elements. After spotting, the DNA is immobilized (either by UV cross-linking or baking), the unused surface is blocked (by succinic anhydride or sodium borate) and, as a final step, the DNA on the slide is denatured (by heat or alkali treatment). Processed slides may be stored dry for several months prior to hybridization.

**Preparation of Probes:**
Initiation of CDNA microarray fabrication necessitates the assortment of suitable probes to be applied. Generally, fluorescent probes are prepared to hybridise to the microarray. These are prepared from messenger RNA from the cells or tissues of interest. Depends on key property, required mRNA can be isolated and extracted from the cells or tissues of interest. These mRNAs employed to synthesized complimentary DNA using reverse transcriptase enzyme. Then synthesized cDNA is labelled by the incorporation of fluorescent or radioactive nucleotides into the DNA. The two samples with contrast property are labelled using two different fluorescent dyes - say, Cy3 (red) or Cy5 (green). These labelled cDNA then hybridised to the microarray slide or DNA chip. Two differentially labelled cDNA mixtures permit the measurement of ratio of fluorescence; this circumvents most of the problems of hybridisation kinetics.

**Potential Applications In Medicinal Plant Study**

Here we have discussed some fundamental of the microarray technology with their analysis and interpretation of the data now we would like to discuss, the major application of this technology, without which meaningful evaluation of gene expression changes would be impossible. The general objectives for plant medicine study can be compiled as need to build IPR & field genebank, and validation of traditional medicine with safety and efficacy for treatment of both human and animal disease. The data generated by using micro array technology can be useful for in-situ conservation of medicinal plants in and around the region of interest. This will can be achieved by using DNA micro array technology. Here we have suggested some potential application of DNA micro array in study of medicinal plants- for Genotyping; Gene Expression; Research into Diseases; Mapping Genomic Libraries

- gene Typing
- Medicinal Gene Expression Profiling
- Genome-wide Studies
- Determination of molecules against drug resistance agents
- Host Gene Expression Profiling during treatment with medicinal plant recipe
- Host Genomic Polymorphism Determination

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