GMR
The Original

A REVIEW ARTICLE: APPLICATIONS OF GENETIC ENGINEERING, RECOMBINANT DNA TECHNOLOGY, AND GENETIC IMPROVEMENT OF MEDICINAL & AROMATIC PLANTS FOR FUTURE PROSPECTS

Dr. Priyansh Saraiya[1*], Dr. Sunita D. Ram[2], Dr. Shubham Mulewa[3], Dr. Shiromani Mishra[4], Dr. Pooja Sahu[5]

[1*]MD scholar, Department of Dravyaguna Vigyana, Government Dhanwantari Ayurved Medical College, Ujjain (M.P.), vaidyapriyanshsaraiya@gmail.com
[2]Professor, Department of Dravyaguna Vigyana, Government Dhanwantari Ayurved Medical College, Ujjain (M.P.), drsunitadram72@gmail.com
[3]MD scholar, Department of Dravyaguna Vigyana, Government Dhanwantari Ayurved Medical College, Ujjain (M.P.), golu307.gm@gmail.com
[4]Professor & HOD, Department of Dravyaguna Vigyana, Government Dhanwantari Ayurved Medical College, Ujjain (M.P.), shiromishra@gmail.com
[5]Assistant Professor, Department of Dravyaguna Vigyana, Om Ayurveda Medical College, Betul, M.P. misthy041024@gmail.com

ABSTRACT
Medicinal and aromatic plants play a crucial role in benefiting humans and other animals directly and indirectly by providing the raw materials for pharmaceutical industries and curing severe diseases. These plants provide natural medicines and have great importance in sustaining the economy of several countries of the world. Herbal therapy has been essential in understanding and treating a wide range of human and animal illnesses throughout history. The chemical compounds found in medicinal plants have the ability to treat a wide range of illnesses. It is increasingly essential to improve the genetic composition of these plant species for both therapeutic and commercial reasons. With the creation of around 5500 plant species, mutation breeding is an important tactic. Chromosome doubling, or polyploidy induction, can produce larger, more valuable plant parts with both medical and commercial use. Plant tissue culture (PTC) is an essential breeding method that produces pharmaceutical secondary metabolites by manipulating the genomes of medicinal plants through Agrobacterium-mediated gene transformation and artificial polyploidy. Defects in first- and second-generation gene editing technologies, which depended on synthetic endonucleases like zinc finger endonuclease (ZFN) and transcription activator-like receptor nuclease (TALEN), have been corrected thanks to the development of the third-generation clustered regularly interspaced short repeats (CRISPR)-Cas9 gene editing system. These gene editing techniques make it easier to modify medicinal plants' secondary metabolite pathways. For medicinal and aromatic plant species that might find it difficult to undergo stable genetic change, virus-induced gene silencing (VIGS), which is based on short interfering RNA-mediated RNA silencing, offers a quick substitute for eliminating gene expression.

KEYWORDS: Gene,Genome, Medicinal plants, Genetic Engineering, RNA & DNA

INTRODUCTION

Food production systems are under the dual pressure of enhancing productivity whilst conserving nature and protecting
George Johann Mendel in 1885 showed that certain hereditary factors operate in all biological species. The Danish biologist Wilhelm Johannsen called these factors genes. It is now known that genes not only transmit hereditary characteristics but also mastermind the entire process of life. The genes are located in chromosomes, which are situated in the nucleus of the cell. It can be said that the genes form the riddle and the chromosomes represent the mystery. Much of the mystery surrounding the genes was cleared up with the discovery of the structure of DNA, announced by JD Watson and Francis Crick in April 1953. The structure of DNA resembles a long rope ladder twisted around like a corkscrew. The two sides of the ladder are long chains of sugars and phosphates in repeated sequences.
DNA contains the bases as follows:

- Adenine - A
- Cytosine - C
- Thymine - T
- Guanine - G.An A will form a bridge only with T and C with G. So the pairs A-T, T-A, C-G, and G-C form, in a way, a four-letter alphabet with which messages can be spelled out. It makes up what is known as the genetic code, which is not only complex but also extensive. In 1977, Fred Sanger pointed out that the DNA code of a virus, when decoded by computer, came to a printout of 15 meters.

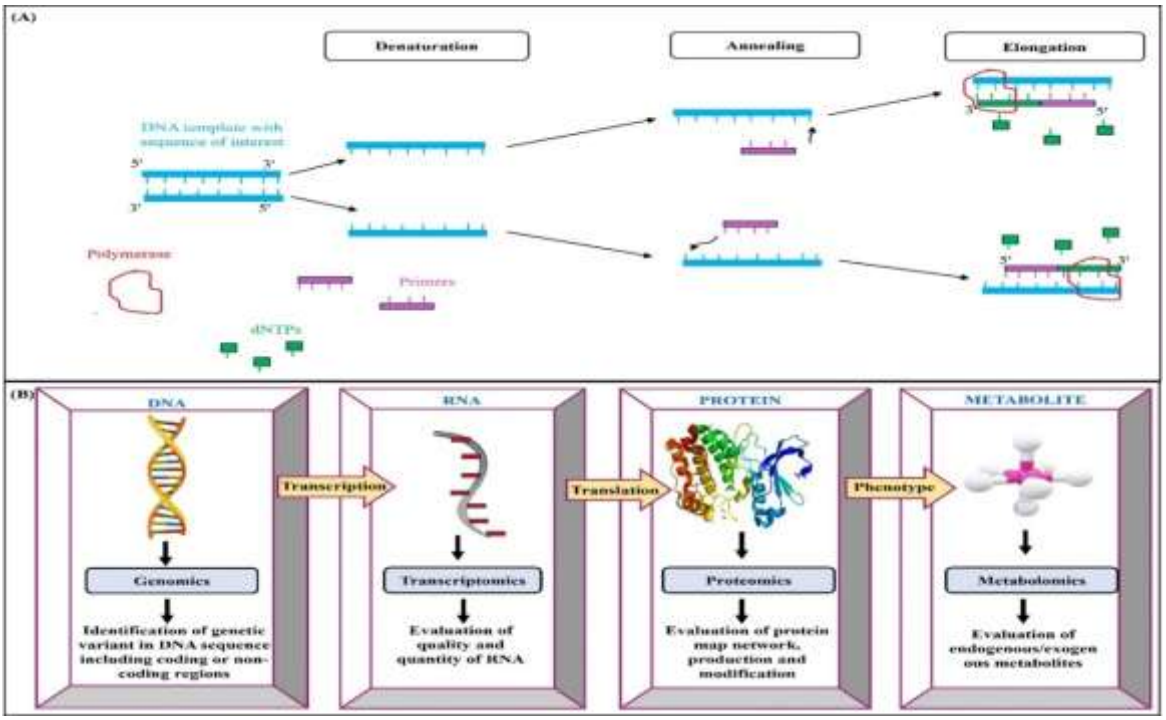

Image no 1: Gene amplification is a biological process in which a specific genomic region or the number of copies of a specific gene can be increased

(A) Steps involved in Polymerase Chain Reaction, including denaturation: DNA unbinding; annealing: primers binding to their complementary DNA sequences; and elongation: making up the replica of the exposed 'template' strand to make a full DNA molecule. (B) Different omics technologies (i.e., genomics for DNA evaluation, transcriptomics for RNA evaluation, proteomics for protein evaluation, and metabolomics for metabolite evaluation) are involved in medicinal plant modifications.

The genes control all functions of cell and body growth. The two main events in the life of most cells are multiplication (by division) and synthesis of proteins. Both these operations are carried out based on blueprints coded in the genes.
Genetics has made valuable contributions to the improvement of plants having economic, medicinal, and ornamental value by applying various techniques like selective breeding. It has an important role in mitosis and meiosis. Mitosis results in precise, equal distribution of chromosomes from a parent nucleus to the daughter nuclei. Further, it maintains equilibrium in the amount of DNA content of cells. Mitosis is associated with vegetative propagation of plants. The somatic number is the number of chromosomes in a plant cell nucleus, which in normal conditions remains constant, but varies with different species. Meiosis occurs in the case of formation of reproductive cells (gametes or meiospores) in sexually reproducing species. Meiosis also has a genetic significance because of the formation of four haploid nuclei from a single diploid one, in two successive divisions, thus balancing the doubling of chromosome number that results from fertilization. During the process of meiosis, crossing over provides new combinations of genetic material and, hence, new combinations of characters in offspring. The process of segregation of chromosomes also occurs in meiosis.
Materials & Methods:
The cultivation and utilization of medicinal plants play a pivotal role in the economic fortune of certain countries, with the raw materials derived from these plants serving as a primary source of income 2.For instance, pharmaceutical compounds like lupeol acetate, isolated from Indian sarsaparilla root extracts 3 are employed in the treatment of various diseases, and serpentine, obtained from the roots of the Indian snake plant is used to reduce blood pressure and treat hypertension. Climate change has had profound effects on higher plants, including medicinal ones, and the risk of extinction for such plants has escalated due to their limited adaptability to changing environmental conditions 4]. Plant domestication represents a dynamic evolutionary process that transforms wild species into valuable cultivated forms through genetic modification 5]. Significant changes occur during the domestication of plants, including the development of larger fruits or grains, stronger plants, reduced seed dispersal and dormancy, decreased bitterness, increased central stem growth, altered photoperiod sensitivity, and synchronized flowering [6]. However, medicinal and aromatic plants have not undergone this domestication process, necessitating plant breeders to address these limitations through faster breeding methods. It is now imperative for researchers to move past any discrimination and leverage modern breeding and biotechnology-based approaches to enhance medicinal and aromatic plants. Plant breeders can now access complete genomic and transcriptomic data for these plants through next-generation sequencing. These genomics and transcriptomics data can be combined with proteomics and metabolomics information to reveal unidentified natural products within medicinal plants, previously [7

Table 1: Medicinal plants and their effective parts in the treatment of human diseases.

Plant Name	Botanical Name	Part Used	Medicinal Uses
Aswagandha/One year	Withania somnifera Family: Solanaceae	Root, Leaves	Restorative tonic, stress, nerve disorder, aphrodisiac.
Chiraita(high altitude)/Oneyear	Swertia chiraita Family: Gentianaceae	Whole Plant	Skin Disease, Burning Sensation, fever
Kakamachi/Within one year	Solanum nigrum Family: Solanaceae	Fruit / whole plant	Dropsy, general debility, diuretic, anti-dysenteric
Gudmar/ madhunasini/Four Fouryear	Gymnema sylvestre Family: Asclepiadaceae	Leaves	Diabetes,hydrocil, asthma.
Longpeeper/ Pippali/Aftertwo tothreeyears	Peeper longum Family: Piperaceae	Fruit,Root	Appetizer, enlarged spleen, bronchitis, cold, antidote

The advances in techniques pertaining to genetic manipulations in plant cells have made a significant impact on improving the yield of medicinal plants.

Mutation

Mutation is represented as variation in characters of the species. It is caused either due to environmental changes or changes in hereditary constitution. Normally, as a response to environmental changes, variations are observed, but the original traits are restored when changes in the environment are also withdrawn or disappear. This type of change and restoration is not heritable and is also not built into the genotype. They are termed only as phenotypic variations and commonly called modifications. It is evident that between organisms of similar genotypes there are phenotypic variations. However, when a change occurs in the genome of an individual that is not caused by the environment, it may make a permanent evolutionary change. This is termed a mutation and represents a sudden change in genotype causing qualitative or quantitative alterations of genetic material. Mutations are of two types, namely chromosomal mutations and point mutations. The former type of mutation is also called chromosomal aberration, which in many cases leads to changes in the amount or position of genetic material. On the other hand, changes in a gene or cistron of a DNA molecule cause point mutations, and they are permanent and heritable.

A mutation that occurs due to some unknown reason from nature is called a spontaneous mutation. This has been observed in some plants, bacteria, viruses, etc. Mutations can also be induced by artificial means with certain reagents called "mutagens" and are called induced mutations. The various mutagens used are exposure to UV rays, X-rays, ionizing radiation, certain chemicals, abnormal environments, etc. The chemical mutagens used are nitrogen mustard, formaldehyde, nitrous acid, ethyl ethane sulfonate, 5-bromouracil, 2-aminopurine, manganese chloride, etc.

The changes caused by mutations include morphological and anatomical changes, as well as changes in the chemical composition of the plants. This is significant for the medicinal plants. In some cases, favourable changes and yields in active constituents of plants have been achieved. Mutations may cause resistance of a medicinal plant towards certain diseases. But, in all these cases, the plant may become susceptible to climatic conditions, certain other diseases, retardation in growth, etc. These undesirable effects are to be eliminated by breeding and selection.

Polyploidy

Polyploidy has exhibited various useful effects on medicinal plants like digitalis, mentha species, poppy, plants containing tropane alkaloids, lobelia, etc. The specific number of chromosomes is a characteristic of each species and is called the genome, which is observed in all types of organisms. The term "euploidy" is a type of ploidy in which the genome contains a whole set of chromosomes, and euploidy includes monoploidy, diploidy, and polyploidy. When the organism contains more than two genomes, it is called polyploidy. Polyploidy occurs in a multiple series of 3, 4, 5, 6, 7, etc. of the basic chromosome or genome number, and then accordingly, it is called triploidy, tetraploidy, pentaploidy, hexaploidy, heptaploidy, and octaploidy, respectively.

Polyploidy is caused through cell division, physical agents like X-rays, centrifugation, temperature shocks, and chemical agents, mainly colchicine, veratrine, sulphanilamide, hexachloro-cyclohexane, and mercuric chloride. The chemical agents cause disturbance to the mitotic spindle of dividing diploid cells and cause non-segregation of already duplicated chromosomes and thus convert diploids into tetraploid cells. The phenomenon of polyploidy is of greater significance to medicinal plants. It may cause formation of new species, adaptability to various habitats, and mainly accumulation of vitamins.

Perhaps the best-known chemical to cause polyploidy is colchicine, an alkaloid obtained from Colchicum species like Colchicum autumnale, C. luteum, and C. speciosum. Colchicines prevent sister chromatids from separating into daughter nuclei at anaphase. These chromatids remain attached by their common centromere in C-metaphase. The chromatids eventually separate, but remain in the same nucleus. An interphase occurs, followed by a second C-metaphase, involving a doubled chromosome complement. Hence, the chromatid pairs are doubled in the second C-metaphase. Likewise, the cell undergoes one, two, or more than two rounds of DNA replication, causing polyploidy. The activity of colchicine mentioned here is caused by its interaction with disulphide bonds of spindle proteins and by inhibition of conversion of

globular proteins to fibrous proteins. The capacity of colchicine to induce polyploidy varies along with its different derivatives. Chemically induced mutations may lead to variations in the biochemical composition of plants. Colchicines treatment given to medicinal plants has shown promising results in many cases. The observations in tropane alkaloids by way of polyploidy are more specific in stramonium, where the yield of the crop is enhanced by 60-150 per cent in the 4n form. The plants like lobelia, cinchona, belladonna, acorus, squill, cannabis, and poppy also show increased yield of respective compounds in the 4n form. Polyploidy may cause a reduction of total glycosides of D. purpurea and D. lanata, but in the latter case raises the contents of lanatosides A and B. The increase in chemical contents may not be coincidental with the phenotype of the medicinal plant. There may also be a size reduction along with enhancement in the content of active constituents. Some plants do not show any change in chemical contents as a response to polyploidy.

Chemodemes (Chemical Races)

The knowledge about plant chemical races has surfaced due to thorough chemical analysis of a huge number of different plants with the help of modern analytical instruments. Chemodemes are regarded as a group of plants of a species that have identical morphological characters, but differ in their chemical nature. Due to this, chemodemes are considered chemically separate groups within a species. The observation of chemodemes can be confirmed only by growing different plants of a species in identical conditions, preferably from the seeds and for many generations. In this way, it shows variations in the type or content of certain constituents, such as secondary metabolites of medicinal importance. The chemical characters of chemotypes are hereditary. Chemical races have been reported in Digitalis purpurea and Digitalis lanata. In the former, the races or strains identified are streptoside, digitoxin, and digipurpurin. These chemical races in D. purpurea yield different proportions of glycosides obtained from digitoxin and gitoxin. Depending on the content of lanatosides A and C, the chemical races in D. lanata are D. lanata Ehrh, chemovarieties A and C. The chemotypes of Rheum palmatum are also known, which yield different values for the thein-chrysophanol ratio. The different varieties of Prunus communis (almond) differ by the presence or absence of amygdalin. Chemical races have also been reported in Claviceps purpurea, Duboisia myoporoides, and Duboisia leichharditii. Three chemotypes are evident in Withania somnifera. Chemotype I contains mainly withaferin; chemotype II has similar compounds, and chemotype III contains withanolides (a mixture of steroidal lactones). Withaferin and withanolides have different medicinal actions.

Examples:

- Cinnamomum zeylanicum
- Ocimum menthaefolium
- Cinnamomum camphora
- Eucalyptus dives
- Ocimum sanctum

The discovery of chemotypes has enabled the pharmacognosists to select high-yielding chemical strains or to eliminate chemical strains which contain toxic principles. The chemotypes are induced by breeding in some species to manipulate active constituents, with a view to enhance the therapeutic efficacy.

ARTIFICIAL MUTATION

Artificial mutations are those which are induced artificially in the living organism by exposing it to an abnormal environment, such as radiation, certain physical conditions like temperature, and chemicals, all of which are called mutagens or mutagenic agents.

(1) Radiation mutations: The electromagnetic waves of short wavelength (ultraviolet light, X-rays, gamma rays, alpha and beta rays) are radiation mutagens. The X-rays and gamma rays are called ionizing radiations, and also include alpha particles, beta rays, thermal and fast neutrons. Due to ionizing radiations, in many cases, a water molecule in a biological system releases one electron and becomes unstable and eventually splits into a hydrogen ion and a hydroxyl radical. The hydrogen ion reacts with O_2 and produces a hydroperoxyl (HO_2) radical. Both these radicals, viz., hydroperoxyl and hydroxyl, are potent oxidizing agents. When chromosomes and their DNA are struck by such radicals, they react, due to which the sugar-phosphate part of DNA may be impaired, leading to chromosomal mutations, like breaks, deletions, additions, inversions, and translocations.

(2) Chemical mutations: Some chemical mutagens, like nitrogen mustard, formaldehyde, nitrous acid, and ethyl ethane sulfonate, alter the chemical constitution of DNA bases and cause transitional substitution in DNA. But some compounds like 2-aminopurine, urethane, 5-bromouracil, caffeine, triazine, phenol, and certain other carcinogens act as a base analog and bring out copy error mutations in DNA. In general, the chemical mutagens have profound cellular effects like production of abnormal DNA (nitrogen mustard), inhibition of deoxyribonucleotide synthesis (deoxyadenosine), and inhibition of cytochrome oxidase with resultant The artificial production of mutations in medicinal plants is an important milestone in the development of cultivation technology.

The higher solasodine content is achieved by applying radiation and chemical mutagens in Solanum khasianum. It is also reported that chemical mutagens have been successfully used in increasing the morphine content of Papaver somniferum. The tuber yield and diosgenin content of Dioscorea bulbifera are increased by radiation. The economically important characters of Atropa belladonna have been enhanced by radiation and chemical mutagens. The agronomic performance and harvest index of Mentha arvensis var. piperascens (Japanese mint) have been improved by exposure to gamma radiation. The effects of artificial mutations have been extensively studied on different species of mentha. The Capsicum seeds (Capsicum annuum) treated with sodium azide and ethyl methane sulphonate have led to plants giving higher contents of capsaicin.

HYBRIDIZATION

The process through which hybrids are produced is called hybridization. A hybrid is an organism which results from the crossing of two species or varieties differing at least in one set of characters. The resultant hybrids are monohybrids (one pair of different characters), dihybrids (two pairs of different characters), or polyhybrids (more than two pairs of different characters). Hybridization helps in inducing in a single variety the favorable characters of other varieties or species, and sometimes producing new and favorable characters which are not present in both the parents.

The hybridization of Withania somnifera Israeli chemotype II and W. somnifera South African chemotype has led to the formation of a new hybrid that contains three new withanolides. Hybrids like Digitalis purpurea x D. lanata and D. purpurea x D. lutea contain the principal glycoside as lanatoside A, along with lanatosides B and E, but are devoid of lanatoside C or purpurea glycoside A. During the cross of Solanum incanum (1.8 per cent solasodine) and S. melongena (traces of solasodine), the first generation bears more fruits (berries) with solasodine up to 0.5 per cent. The second generation has proved to be a high-yielding source for solasodine.

A recent development in hybridization is through the medium of tissue culture. The protoplast cultures are employed for this purpose. Plant protoplasts are cells without cell walls. Such protoplasts in cultures can be fused (protoplast fusion or asexual hybridization). The fusion can be arranged in cells of the same origin or between different species.

Plant tissue culture (PTC);

Plant Tissue Culture (PTC) has a vital role in the conservation of biodiversity, especially for both common and endangered medicinal plant species, as stated in a report by the World Health Organization. About 80% of the global population relies on herbal remedies for health concerns [62,63]. Tissue culture allows for mass propagation and germplasm conservation of these plants in a short time and with limited space. In the field of plant biotechnology, in-vitro culture is a pivotal tool that leverages the remarkable totipotent nature of plant cells [13. Somatic embryogenesis is a process where a bipolar structure similar to a zygotic embryo is formed by a non-zygotic cell, which can develop into a new plant 13]; Von Arnold et al., 2003). Successful plant regeneration through somatic embryogenesis has been achieved in medicinal plants such as Aloe [14] and Vitis vinifera [15]. The technique of protoplast culture has also been used to generate medicinal plants, including Artemisia judaica L. and Echinops spinosissimus Turra [15]. Various culture media have been developed based on plant culture, including the Murashige and Skoog (MS) medium [14], Schenk and Hilderbrandt (SH) medium [12], and Woody Plant Medium (WPM) [13]. The MS medium is commonly used for culturing medicinal plants. For instance, studies have shown that 100% seed germination of Acorus calamus is possible using the MS medium [14]. The morphogenetic responses of seedlings can vary depending on the concentrations of cytokinins and auxins in the MS medium. In Aristolochia indica Linn., indirect organogenesis was observed using axillary shoots 25]. When the MS medium was supplemented with kinetin (KN) and 6-benzylaminopurine (BAP), a 95% callus induction rate was recorded, and after subculturing the calli on the same medium with these supplements, better shoot regeneration rates were observed.

Several methods like media optimization, bioinformatics, elicitation, Agrobacterium transformation, and scale-up [15] directly depend on or are integrated with plant tissue culture (PTC) to enhance the valuable production from medicinal and aromatic plants in in-vitro condition. Plant tissue culture is also a platform for the remarkable strategy of hairy root culture, which is used in the synthesis of valuable secondary metabolites from medicinal and aromatic plants, with higher biochemical and genetic stability compared to conventional in-vitro cultures [16]. Balasu Bramanian et al. [17] reported enhanced phenolic flavonoid and quercetin content in radish plants induced by hairy root culture mediated through Agrobacterium rhizogenes. The advantage of the hairy root culture has also been reported in enhanced production of flavonoids, phenolic acids, and wedelolactone content in Sphagneticola calendulacea [18], and also promotes the higher production of tropane alkaloids of hyoscyamine and scopolamine in Scopolia lurida [19]. The Agrobacterium-mediated hairy root culture is the platform for large-scale production of secondary metabolites using elicitors [20], bioreactors [21, artificial polyploidy [22], and genome editing of CRISPR-Cas9 [23].

GENETIC ENGINEERING AND RECOMBINANT DNA TECHNOLOGY

The method of artificial synthesis of new genes and their subsequent transplantation into the genome of an organism, or the method of correcting defective genes of an organism by molecular biological techniques, forms the discipline called genetic engineering.

Recombinant DNA technology involves gene splicing to change the characteristics of plants and animals by implanting in them genes from other organisms and, in some cases, even from other species.

The genetically engineered products that already exist in the market are human growth hormone, human insulin, alpha-interferon, and the hepatitis-B vaccine, and the products coming in the next few years are as follows:

1. Interleukin-2, to fight certain types of cancer
2. Beta-endorphins for the treatment of pain
3. TPA (tissue plasminogen activator for treating heart attacks)
4. Somatostatin for treatment of pituitary diseases
5. Erythropoietin, or EPO, is designed for treating anemia in patients undergoing kidney dialysis.

The secondary metabolites from plants, which are expected to give promising results by genetic engineering, include

1. Cardiac glycosides.
2. Morphine alkaloids.
3. Stigmasterol or diosgenin, which give steroid hormones and corticosteroids.

Transgenic Plants

Application of genetic engineering techniques for the improvement of plants is a fast-developing area of research. The advent of modern techniques of genetic modification has enabled the removal of individual genes from one species and insert them into another, without the need for sexual compatibility. Once the new gene has been inserted into the plant, offspring that will contain copies of the new gene can be produced traditionally.

Transgenic plants carry the stably integrated foreign genes. It is important that the new DNA remains stable and can pass the new gene to its offspring as well. The introduction of foreign genes is done by genetic transformation (gene transfer), also called Transfection. It is carried out to achieve the same purpose as viral or chemical transformation, by which a stable or transient change is obtained in the cells. But it is accomplished with the help of DNA that is tailored as per the needs/aims.

Transgenic plants possess a wide variety of potential uses, among which crop improvement is most significant. It includes goals like herbicide resistance, insect resistance, changes in oil and protein contents of the crops, enhanced quantities of products, etc. The overall transformation process includes the following steps:

- Gene transfer.
- Selection of transgenic plants.
- Recovery of transgenic whole plants.

Gene transfer or DNA delivery systems are of the following types:

(1) Vector-mediated: Using Agrobacterium tumefaciens
Using viral vectors.
Main Flat Biotechnology

(2) Vector-free (Direct Gene Transfer)
Electrical methods (Electroporation)

Physical methods

Microinjection, silicon carbide whiskers, microprojectile bombardment (biolistic gene transfer), and sonication.

Chemical methods

Using polyethylene glycol (PEG), fusion of protoplasts with liposomes.

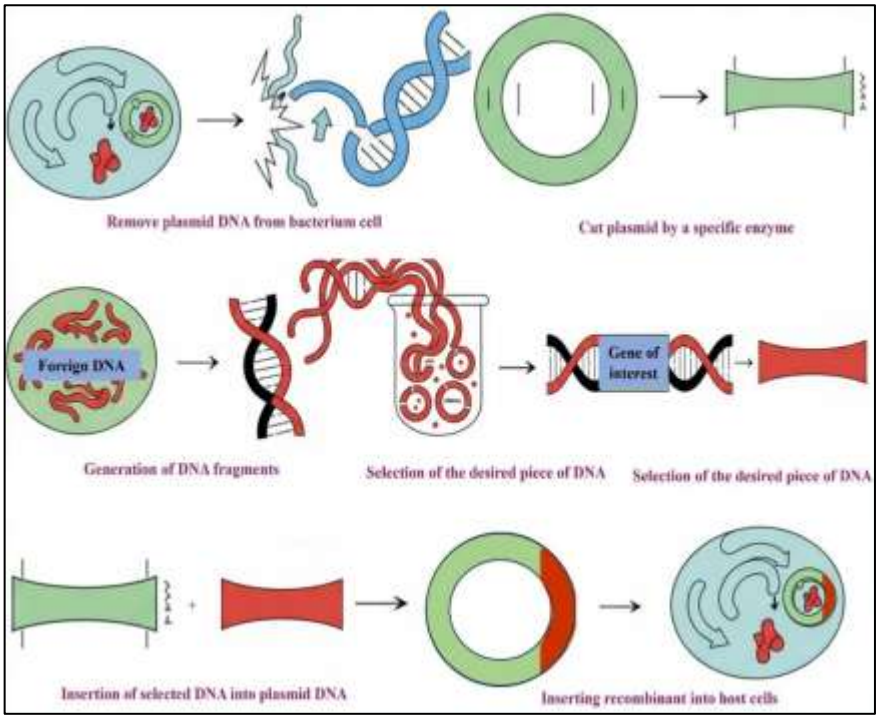

Image no. 2 showing the different steps involved in recombinant DNA technology to manipulate and combine DNA segments from different sources to produce a new genotype of medicinal plants.

Gene Transfer

(a) By Using Agrobacterium Tumefaciens

This Gram-negative bacterium commonly occurs in soil and causes 'crown gall disease' in higher plants. It enters the plant through the mechanically wounded tissue site. This infection causes local proliferation of undifferentiated tissue (tumorous outgrowth). Agrobacterium tumefaciens possesses a special ability to transfer some of its DNA into the nuclear DNA of an infected host plant because of functions encoded on its "Ti" (tumour-inducing) plasmid. The transferred DNA, or X-DNA, of this plasmid encodes substances that act as plant hormones, causing the proliferation of tissue seen in the infected plant. The Ti plasmid of this bacterium has been modified by means of genetic engineering to create "disarmed" plasmids (also called vectors), which can carry any DNA sequence into A. tumefaciens-infected plants without tumorous

growth on the host plant. These cloning vectors derived from the Ti plasmid have been used for replication in both A. tumefaciens and Escherichia coli bacterial strains. This makes it possible to clone foreign DNA sequences into the vectors using an E. coli host, as well as the further transfer of the completed vector to an A. tumefaciens host.

(b) By Electroporation
This technique is based on the use of short electrical pulses of high field strength to promote DNA uptake by protoplasts. The optimal electroporation conditions may vary in different plant species. Buffers greatly influence the gene transfer efficiency and also the protoplast survival rate.

(c) By Using Microinjection
Microcapillaries and microscopic devices are used to deliver DNA into protoplasts. It is considered one of the most precise techniques. However, it is a very skilled process, and expensive equipment is required.

(d) Biolistic Gene Transfer
The DNA is bombarded into the intact cell using a biolistic device such as a particle gun. It can only be used for intact tissues. Because pressure is to be applied, it is not suitable for protoplasts. Like microinjection, this is also a very precise technique.

(e) Silicon Carbide Whiskers
Silicon carbide forms long, needle-shaped crystals (whiskers) in solutions like polyethylene glycol, 1-3 percent. When cells are vortex mixed in the presence of whiskers and DNA, the DNA can be introduced into the cells following penetration by whiskers.

Gene Transfer by Using Chemical Agents
Various chemical treatments have been used to stimulate DNA uptake by protoplasts. Among them, polyethylene glycol is the most common. It acts by increasing the permeability of cell membranes. The optimal concentrations of PEG are 15-20 percent.
Tobacco was the first plant to be genetically transformed in 1983, with cereals beginning in 1990. Recently, genetically modified soy has reached the market.
Most cereal food crops in Western countries have been made insect- and pest-resistant by incorporating suitable genes. Owing to this, the percentage spoilage of crops per annum has declined sharply and reduced. Consequently, the use of these harmful chemicals has also been drastically reduced, proving to be beneficial to the environment.

Transgenic Plants as a Source of Vaccines
- It has been found that developing nations are still deprived of modern vaccines. This is mainly because of a lack of refrigeration and other equipment needed for such programs. It has been shown that plants can be utilized for preparing vaccines, given that they can be induced to synthesize appropriately folded bacterial and viral proteins that stimulate an immune response.
- A specific DNA sequence encoding the expression of a surface antigen of a pathogen is isolated and ligated to a promoter (e.g., the Cauliflower Mosaic Virus 35S promoter), which can regulate the production of the surface antigen in a transgenic plant. This gene is then transferred to plant cells using a procedure that results in its integration into the plant genome, such as through the use of an Agrobacterium tumefaciens plasmid vector system.
- A plasmid containing the hepatitis B surface antigen gene, a promoter, a marker gene, and flanking T-DNA sequences has been prepared and used in A. tumefaciens.

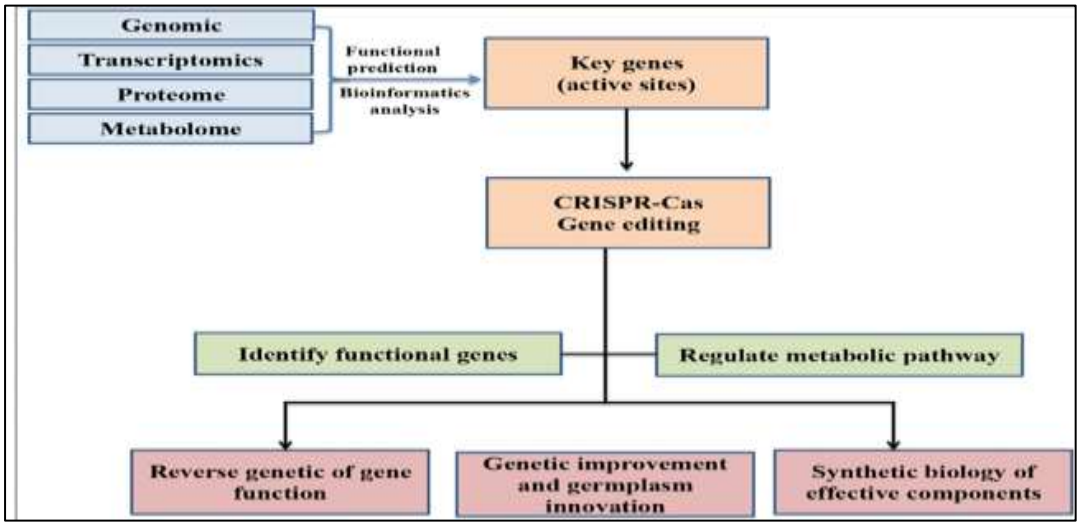

Diagram for CRISPR/Cas gene editing strategies of medicinal and aromatic plants combined with omics technologies

Future implication

Approximately 500,000 plant species have not yet been fully investigated for their possible therapeutic qualities, but the future of medicinal plants is bright. These plants' unrealized therapeutic potential may be vital to present and future medical studies. The development of human civilization, including religious and ceremonial customs, has been greatly aided by medicinal herbs. In the near future, genetic engineering and mutations will be crucial tools for plant breeders. In the past, direct selection and mutations have been used to create several plant kinds. A new and broad paradigm for the use of mutant breeding for crop improvement has been created as a result of recent advancements in in vitro culture and molecular tools. Because of its broad range and high mutation frequency, heavy ion beam irradiation has become a popular technique for causing mutations in a variety of plant species. The Food and Agriculture Organization (FAO) has formally recognized 3248 mutant cultivars, demonstrating the influence of induced mutations on crop improvement. To improve crop species, modest amounts of plant tissues and callus can be exposed to mutagenesis using in vitro culture procedures. The advancement of in vitro cell selection methods for disease resistance will also be essential in the future.

CONCLUSION

The world's natural heritage of aromatic and therapeutic plants helps some nations maintain their economies. Damage to such natural heritage can have an impact on a nation's economy and citizens' health, and some nations depend on aromatic and therapeutic plants for industrial uses. Many nations throughout the world began producing chemical compounds on a massive scale in order to maintain their prosperity and address major health issues. The medicinal and aromatic plants that have declined due to overexploitation and environmental degradation are limited by plant breeding techniques. Without coordinated efforts to protect biodiversity, these precious resources will continue to run out. Compared to other plants, medicinal plants received less attention from breeders; as a result, these plants produced low-quality and low-productivity compounds. Recent developments in plant breeding techniques have improved aromatic and therapeutic plants by revealing the complexity of chemical compounds. A number of medicinal and aromatic plants can have their genomes altered using gene editing techniques like CRISPR-Cas9 to produce useful pharmaceutical compounds. Similar to this, plant tissue culture (PTC) and artificial polyploidy induction are crucial for producing large, medicinally valuable plant parts and for regenerating entire plants from the callus to support aromatic and medicinal plants, respectively. Additionally, it should become standard practice in plant breeding programs to gather and preserve medicinal and aromatic germplasm.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest: Authors declare that there is no conflict of interest between them in this research.

Declaration of competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

REFERENCES

1.K.Y. Alain, A.N. Tamfu, S. Kucukaydin, O. Ceylan, A.D.C. Pascal, A. Felicien, S.C.K. Dominique, M.E. Duru, R.M. Dinica, Phenolic profiles, antioxidant, quorum-sensing, antibiofilm, and enzyme inhibitory activities of selected Acacia species collected from Benin, Lebensm. Wiss. Technol. 171 (2022) 114162.

2. K.C. Ugoeze, Phytopharmaceuticals for treating sexually transmitted diseases. Herbal Drugs for the Management of Infectious Diseases, 2022, pp. 179–261.

3. I. Chatterjee, A.K. Chakravarty, A. Gomes, Daboia russellii, and Naja kaouthia venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla Hemidesmus indicus R. Br., J. Ethnopharmacol. 106 (1) (2006) 38–43.

4. M. Halder, S. Jha, The current status of population extinction and biodiversity crisis of medicinal plants is in Medicinal Plants: Biodiversity, Biotechnology and Conservation, Springer Nature Singapore, Singapore, 2023, pp. 3–38.

5. D.L. Van Tassel, O. Tesdell, B. Schlautman, M.J. Rubin, L.R. DeHaan, T.E. Crews, A. Streit Krug, New food crop domestication in the age of gene editing: genetic, agronomic, and cultural change remain co-evolutionarily entangled, Front. Plant Sci. 11 (2020) 789, https://doi.org/10.3389/fpls.2020.00789.

6.J.F. Doebley, B.S. Gaut, B.D. Smith, The molecular genetics of crop domestication, Cell 127 (7) (2006) 1309–1321, https://doi.org/10.1016/j.cell.2006.12.00 6.

7. M. Niazian, Application of genetics and biotechnology for improving medicinal plants, Planta 249 (2019) 953–973, https://doi.org/10.1007/s00425-019-03099 1

8.S.A. Wilson, S.C. Roberts, Recent advances towards development and commercialization of plant cell culture processes for the synthesis of biomolecules, Plant Biotechnol. J. 10 (3) (2012) 249–268, https://doi.org/10.1111/j.1467 7652.2011.00664.x.

9.T. Ahsan, N. Amjad, A. Iqbal, A. Javed, A review: tissue culturing of important medicinal plants, International Journal of Water Resources and Environmental Sciences 2 (4) (2013) 76–79.

10. G. Garro-Monge, A.M. Gatica-Arias, M. Valdez-Melara, Somatic embryogenesis, plant regeneration and acemannan detection in aloe (Aloe barbadensis Mill.), Agron. Costarric. (2008).

11. T. Murashige, F. Skoog, A revised medium for rapid growth and bio assays with tobacco tissue cultures, Physiol. Plantarum 15 (3) (1962) 473–497, https:// doi.org/10.1111/j.1399-3054.1962.tb08052.x.
12. R.U. Schenk, A.C. Hildebrandt, Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures, Can. J. Bot. 50 (1) (1972) 199–204, https://doi.org/10.1139/b72-026.
13. G. Lloyd, B. McCown, Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture, Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture 30 (1980) 421–427.
14. J.H. Lee, T.H. Han, Micropropagation of the plantlets derived from seeds in the genus Acorus (A. calamus and A. gramineus), Horticulture, Environment, and Biotechnology 52 (2011) 89–94, https://doi.org/10.1007/s13580-011-0154-0.
15. A. Matkowski, Plant in vitro culture for the production of antioxidants—a review, Biotechnol. Adv. 26 (2008) 548–560, https://doi.org/10.1016/ j.biotechadv.2008.07.001.
16. D. Roychowdhury, M. Halder, S. Jha, Agrobacterium rhizogenesmediated transformation in medicinal plants: genetic stability in long-term culture, in: S. Jha (Ed.), Transgenesis and Secondary Metabolism. Reference Series in Phytochemistry, Springer, Cham, 2016, https://doi.org/10.1007/978-3-319 27490-4_8-1.
17. M. Balasubramanian, M. Anbumegala, R. Surendran, M. Arun, G. Shanmugam, Elite hairy roots of Raphanus sativus (L.) as a source of antioxidants and favonoids, 3 Biotech 8 (2018) 128, https://doi.org/10.1007/s13205-018-1153-y.
18. S. Kundu, U. Salma, M.N. Ali, A.K. Hazra, N. Mandal, Development of transgenic hairy roots and augmentation of secondary metabolites by precursor feeding in Sphagneticola calendulacea (L.) Pruski, Ind. Crop. Prod. (2018), https://doi.org/ 0.1016/j.indcrop.2018.05.009.
19. X. Lan, J. Zeng, K. Liu, F. Zhang, G. Bai, M. Chen, Z. Liao, L. Huang, Comparison of two hyoscyamine 6β-hydroxylases in engineering scopolamine biosynthesis in root cultures of Scopolia lurida, Biochem. Biophys. Res. Commun. 497 (2018) 25–31, https://doi.org/10.1016/j.bbrc.2018.01.173.
20. A. Kastell, M. Schreiner, D. Knorr, C. Ulrichs, I. Mewis, Infuence of nutrient supply and elicitors on glucosinolate production in E. sativa hairy root cultures, Plant Cell Tissue Organ Cult. 132 (2018) 561–572, https://doi.org/10.1007/s11240-017 1355-8.
21. N. Patra, A.K. Srivastava, Mass production of artemisinin using hairy root cultivation of Artemisia annua in bioreactor, in: A. Pavlov, T. Bley (Eds.), Bioprocessing of Plant in Vitro Systems. Reference Series in Phytochemistry, Springer, Cham, 2017, https://doi.org/10.1007/978-3-319-32004-5_20-1.
22. E. Dehghan, S.T. H€ akkinen, K.M. Oksman-Caldentey, F.S. Ahmadi, Production of tropane alkaloids in diploid and tetraploid plants and in vitro hairy root cultures of Egyptian henbane (Hyoscyamus muticus L.), Plant Cell Tissue Organ Cult. 110 (2012) 35–44, https://doi.org/10.1007/s11240-012-0127-8.
23. B. Li, G. Cui, G. Shen, Z. Zhan, L. Huang, J. Chen, X. Qi, Targeted mutagenesis in the medicinal plant Salvia miltiorrhiza, Sci. Rep. (2017), https://doi.org/ 10.1038/srep43320.
24. C. Morgana, R. Di Lorenzo, F. Carimi, Somatic embryogenesis of Vitis vinifera L.(cv. Sugraone) from stigma and style culture, Vitis 43 (4) (2004) 169–173.

25.N.A. Siddique, M.A. Bari, M.M. Pervin, N. Nahar, L.A. Banu, K.K. Paul, M.H. Kabir, A.K.M.N. Huda, K.M.K.B. Ferdaus, M.J. Hossin, Plant regeneration from axillary shoots derived callus in Aristolochia indica Linn. an endangered medicinal plant in Bangladesh, Pakistan J. Biol. Sci. 9 (2006) 1320–1323.
26. Z.G. Pan, C.Z. Liu, S.J. Murch, M. El-Demerdash, P.K. Saxena, Plant regeneration from mesophyll protoplasts of the Egyptian medicinal plants Artemisia judaica L. and Echinops spinosissimus Turra, Plant Sci. 165 (4) (2003) 681–687, https:// doi.org/10.1016/S0168-9452(03)00220-6.
27. P. Mignard, C. Font i Forcada, R. Gimenez, M.A. Moreno, Population structure and association mapping for agronomical and biochemical traits of a large Spanish apple germplasm, Plants 12 (6) (2023) 1249.
28. A.N.D.I. Salamah, R. Oktarina, E.A. Ambarwati, D.F. Putri, A. Dwiranti, N. Andayani, Cromosome numbers of some Asteraceae species from Universitas Indonesia campus, Depok, Indonesia, Biodiversitas Journal of Biological Diversity 19 (6) (2018) 2079–2087.
29. S.A. Ceasar, V. Rajan, S.V. Prykhozhij, J.N. Berman, S. Ignacimuthu, Insert, remove or replace: a highly advanced genome editing system using CRISPR/Cas9, Biochim. Biophys. Acta Mol. Cell Res. 1863 (9) (2016) 2333–2344, https:// doi.org/10.1016/j.bbamcr.2016.06.009.
30. J.A. Doudna, E. Charpentier, The new frontier of genome engineering with CRISPR-Cas9, Science 346 (6213) (2014) 1258096.
31.J. Lozano-Juste, S.R. Cutler, Plant genome engineering in full bloom, Trends Plant Sci. 19 (5) (2014) 284–287, https://doi.org/10.1016/j.tplants.2014.02.014.
32. L. Liu, X.D. Fan, CRISPR–Cas system: a powerful tool for genome engineering, Plant Mol. Biol. 85 (3) (2014) 209–218, https://doi.org/10.1007/s11103-014 0188-7.
33. M.M. Mahfouz, A. Piatek, C.N. Stewart Jr, Genome engineering via TALENs and CRISPR/Cas9 systems: challenges and perspectives, Plant Biotechnol. J. 12 (8) (2014) 1006–1014, https://doi.org/10.1111/pbi.12256.
34. H. Xu, J. Song, H. Luo, Y. Zhang, Q. Li, Y. Zhu, J. Xu, Y. Li, C. Song, B. Wang, W. Sun, Analysis of the genome sequence of the medicinal plant Salvia miltiorrhiza, Mol. Plant 9 (6) (2016) 949–952.
35. Z. Niu, F. Zhu, Y. Fan, C. Li, B. Zhang, S. Zhu, Z. Hou, M. Wang, J. Yang, Q. Xue, W. Liu, The chromosome-level reference genome assembly for Dendrobium officinale and its utility of functional genomics research and molecular breeding study, Acta Pharm. Sin. B 11 (7) (2021) 2080–2092, https://doi.org/10.1016/ j.apsb.2021.01.019.

36. S. Gao, B. Wang, S. Xie, X. Xu, J. Zhang, L. Pei, Y. Yu, W. Yang, Y. Zhang, A high quality reference genome of wild Cannabis sativa, Horticulture research 7 (2020).
37. L. Guo, T. Winzer, X. Yang, Y. Li, Z. Ning, Z. He, R. Teodor, Y. Lu, T.A. Bowser, I.A. Graham, K. Ye, The opium poppy genome and morphinan production, Science 362 (6412) (2018) 343–347, https://doi.org/10.1126/science.aat4096.
38.A.K. Kiss, J.P. Piwowarski, Ellagitannins, gallotannins and their metabolites-the contribution to the anti-inflammatory effect of food products and medicinal plants, Curr. Med. Chem. 25 (37) (2018) 4946–4967.
39.J. Ren, L. Fu, S.H. Nile, J. Zhang, G. Kai, Salvia miltiorrhiza in treating cardiovascular diseases: a review on its pharmacological and clinical applications, Front. Pharmacol. 10 (2019) 753, https://doi.org/10.3389/fphar.2019.00753.
40. Z. Zhou, H. Tan, Q. Li, J. Chen, S. Gao, Y. Wang, W. Chen, L. Zhang, CRISPR/Cas9 mediated efficient targeted mutagenesis of RAS in Salvia miltiorrhiza, Phytochemistry 148 (2018) 63–70, https://doi.org/10.1016/ j.phytochem.2018.01.015.
41. R. Ren, J. Gao, D. Yin, K. Li, C. Lu, S. Ahmad, Y. Wei, J. Jin, G. Zhu, F. Yang, Highly efficient leaf base protoplast isolation and transient expression systems for orchids and other important monocot crops, Front. Plant Sci. 12 (2021) 626015, https:// doi.org/10.3389/fpls.2021.626015.