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Original Article

Effects of Biotechnology on the Conservation of Threatened Medicinal Plants

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Abstract *- Plants native to Asia are today considered to be among the most vulnerable in the world because of different shifts in land use that have occurred over the past two centuries. These shifts in land use have included the transformation of agricultural and natural areas into artificial surfaces. Industrialization, urbanization, and shifts in people's attitudes about how land should be used all contributed to these developments. In certain countries, the status of greater than 66 percent of the world's surviving habitat types is that of an endangered species. Eighty-three percent of all threatened plant species are threatened primarily because of actions carried out by humans. Destruction and habitat loss are additional concerns simply since they induce greater habitat disruption, thereby isolating plant communities. This is a problem since both factors contribute to the destruction and loss of habitat. This makes the situation worse than it already was. On the other hand, there has been an explosion of interest in "nutraceuticals" (also known as "functional foods") during the past 10 years. Nutraceuticals are foods that contain phytochemical components that may have health-promoting or therapeutic characteristics over the long term. Even though it can be difficult to draw a line between medicinal plants and nutraceuticals at times, the primary difference between the two lies in the evidence that long-term use of foods containing health supplements can provide health advantages to the user. Medicinal plants, on the other hand, are used to treat specific medical conditions (this is known as chemoprevention). On the other hand, some medicinal plants have specific medical benefits, despite the fact that they do not contribute anything to the human diet from a nutritional standpoint. These plants can be used as a solution to particular health difficulties for either a short or a long length of time, depending on the situation. There is an evident interest in traditional and alternative medicine all over the world, according to the World Health Organization. At the same time, there is an increasing application of herbs in medical processes (WHO). The use of medicinal plants is a tradition that dates back hundreds of years; yet, in current times, it has grown into a highly lucrative industry that can be found on marketplaces all over the world. In recent times, a vast assortment of herbal products, such as patented pharmaceutical goods, Natural supplements, such as herbal teas, extracts, and essential oils, have been made widely accessible to consumers.*

Keywords *- Biotechnology, Conservation, Endangered medicinal Plants, and in vitro culture.*

1. Introduction

Plants native to Asia are among the world's most threatened due to human activity during the past two centuries, including industrialization, urbanization, and changes in land use that changed agricultural and natural regions to the artificial surface. These changes have occurred because of a combination of factors. In certain nations, the status of more than two-thirds of the world's extant habitat types is categorized as endangered. Eighty-three percent of plant species that are in danger are in danger, mostly because of human activities. Destruction and loss of habitat are additional problems because they contribute to the fragmentation of the remaining habitat, further separating plant populations from one another (Report of European Commission 2008).

On the other hand, within the past ten years, there has been an explosion of interest in "nutraceuticals" (also known as "functional foods"), which are foods that include photochemical components that may have health-enhancing or curative properties over the long run. In spite of the fact that the line Although there is some fuzziness between medicinal plants and nutraceuticals, the main difference is that nutraceuticals are used as food, and their health advantages may be the consequence of prolonged usage as food (i.e. chemoprevention) [19]. On the other hand, many medicinal plants provide unique medical advantages even if they do not play a nutritional function in the diet of humans. These plants can be employed in response to certain health concerns throughout the course of either a short or a long period of time [9].

According to the World Health Organization, not only is there an undeniable interest in traditional and alternative medicine around the globe [26], but there is also a concurrent rise in the utilization of herbs in various forms

of medical practice (WHO). In modern times, the practice of using medicinal plants, which dates back hundreds of years, has evolved into a highly lucrative industry in markets all over the world. A plethora of herbal products, such as patented medicines, food additives, herbal teas, extracts, essential oils, and so on, have lately entered the market [20-23].

The market for medicinal products that are based on plants, as well as herbs themselves, is growing in size all over the world. A decade ago, the revenue generated by the sale of medicinal plants in the North American market reached around $3 billion per year [22]. Brazil is remarkable in South American commerce with 160 million USD for 2007, while China is in the top trade position in Asia with 14 billion USD for 2005, etc. [15]. A rise of the same magnitude was seen in Western Europe, where the total income for the two years spanning 2003 and 2004 was equivalent to six billion US dollars. From 1999 to 2001, there was a 22% rise in sales in the Czech Republic, while there was a 100% increase in sales in Bulgaria [44-45].

The world's flora has a priceless resource in the form of medicinal plants. There are around 2.5 billion higher plant species on Earth. It is estimated that more than 80 thousand of these species have some sort of medicinal use, and somewhere in the neighbourhood of five thousand of these species have specialized therapeutic value. Raw materials are sourced from more than 50,000 different plant species for use in both conventional allopathic treatment and cutting-edge Phytotherapy [44]. About two-thirds of the fifty thousand plants used in the pharmaceutical business are gathered from their natural environments [10]. Only a fraction, between 10 and 20 percent, of the plants that go into medicinal concoctions are grown in fields or other types of controlled environments [42]. The exploitation of natural resources, which has been going on for centuries, and a tremendous rise in interest in the topic pose a serious danger to biological variety. It is possible for poor management of harvesting to lead to the extinction of endangered species or for insufficient farming methods to contribute to the devastation of natural resources. The decline of natural populations, the loss of genetic variation, the extinction of local populations of numerous species, and the degradation of their natural habitats have all been documented by scientific research [34]. This serious problem calls into question whether or not extra attention should be devoted to safeguarding plant populations and current information on how to make better use of these plants. It is crucial to put in extra work to ensure the survival of the plant populations and to have the most recent information on how to make better use of them [45].

Inadequate acreage for cultivation, inadequate harvest management, and excessive harvesting have all contributed to the degradation of natural resources and the loss of biodiversity as the global herb trade has grown steadily over the past few decades. As reported by the United Nations' Food and Agricultural Organization (FAO), annual irreversible losses in the world's flora threaten the stability of the natural resource balance and the ecological system as a whole [10]. At the turn of the century, between four thousand and ten thousand different medicinal species were at risk of going extinct [10]. In order to put an end to the violence that is being committed against nature, efforts should be directed not only toward the preservation of plant populations but also toward the elevation of the level of knowledge necessary for the sustainable utilization of these plants in the allopathic, alternative, and traditional forms of medicine [45].

2. The Essence of Tissue Culture

Procedures and techniques relevant to plant cells were initially employed in the context of basic scientific inquiries in the early 1960s of the previous century. Modern plant biotechnology relies on the plant cell's inherent totipotency [6]. During the procedure of returning to its early embryogenic or meristematic stage, a differentiated cell undergoes dedifferentiation, which is fundamental to the de novo reconstruction of an organism from a single cell. At this point, cells are dividing and potentially developing into either callus tissue that has not undergone differentiation or new tissue, organs, or perhaps an entirely new creature. There are two main ways to accomplish morphogenesis in a petri dish: organogenesis, in which many cells work together to produce an organ from scratch; and (ii) somatic embryogenesis, in which a whole creature is formed from a single cell.

3. Micropropagation

Plants can be propagated vegetatively in vitro (in glass containers under controlled settings) using micropropagation, which ultimately yields several seedlings from the removed tissue while restoring the original plant's genetic potential. Inducing axillary or adventitious shoots often requires the use of tissues rich in meristematic cells. However, it is also possible to induce somatic embryos from differentiated cells. In order to get a big number of plants of a good grade, micropropagation is a technique commonly employed for many different kinds of plants. It is most often used for agricultural plants, as well as vegetable and decorative species, and only to a somewhat lesser amount for plantation crops. Micropropagation, as opposed to clonal propagation, allows for rapid, large-scale propagation of novel genotypes, the use of minimal amounts of original germplasm (especially during the early breeding and transformation stage, when only a few plants are available), and the production of pathogen-free propagules. This is one of the significant advantages of micropropagation over traditional clonal propagation. [1]. When compared to the other subfields of in vitro technologies, clonal propagation has been shown to be of the greatest economic and market importance in the industry. This includes the pharmaceutical industry, which is experiencing consistent growth in demand for raw materials derived from medicinal plants. It provides a method that is both quicker and more diverse for the production of raw materials. On the other hand, it helps solve difficulties caused by the scarcity of natural resources.

At the moment, there is a very large list of research groups all over the world looking into hundreds of different kinds of medicinal plants. In order to be successful with many of these species, various techniques and recipes have been devised. There is not, however, a single methodology that can be applied to each and every species, ecotype, and explant tissue. On the other hand, all of this extensive ongoing research into standardizing explant sources, media composition and physical state, environmental conditions, and acclimatization of in vitro plants have yielded a wealth of information that is continuously expanded upon and provides a solid foundation for the creation of effective protocols for a wider range of species. Expanding the practical use of micropropagation requires a reduction in costs sufficient to make it competitive with seed production or other traditional means of vegetative propagation (such as cuttings, tubers, bulbs, and grafting) [1].

4. Genetic Manipulations

Indirect and direct genetic transformation are two more methods for boosting the amount of physiologically active compounds in plants. Genetic engineering refers to a variety of processes and procedures used to alter the genome in order to produce cells and creatures with enhanced properties or endowed with desired features. Better yield or resistance, as well as increased metabolite production or the synthesis of important physiologically active compounds, may be referred to in these [2]. When isolated desirable DNA pieces are introduced into the cell, often by an electrical field or adhesion, gene transfer may occur directly. In the case of medicinal herbs, this strategy is less common. The isolated genes of interest are transferred, and specific metabolic pathways are activated in plants by indirect genetic transformation using DNA vectors that are naturally present in plant pathogens [7]. Dicotyledonous plants' roots develop "hairs" as a result of *Agrobacterium rhizogenes*. Genetically engineered "hairy" roots create novel, frequently low-content compounds. Hairy roots are a potentially very productive source of important secondary metabolites required for the pharmaceutical sector because of their genetic stability [3]. The manipulations and optimization of the modified hairy roots' production are often the same as for the other in vitro culture methods [41]. Additionally, they are influenced by factors like species, ecotype, explant, nutritional medium, growth circumstances, etc. [12]. All of these potential applications of plant cell division and regeneration principles to actual plant development and further manipulations hinge on the availability of reliable in vitro cultures, whose reliability depends on a wide variety of various conditions.

5. Factors Influencing Cell Growth in vitro.

It was confirmed by the laborious empirical work of in vitro investigations that the genotype of the plant, as well as the mother or donor plant, the explant, and the growth regulators, have the greatest impact on the plant cell's capacity to realize its totipotency and become a fully differentiated plant [29]. In this section, some of the particular and essential conditions that must be met to develop in vitro cultures of medicinal plants will be discussed to facilitate an understanding of the work that must be done and the uniqueness of some concepts.

5.1. Genotypes

The extent to which the genotype influences the morphogenetic potential of excised tissue that is subsequently cultured *in vitro* is quite high [13]. Genetic analysis reveals that diverse plant groupings, including medicinal plants, exhibit a variety of organ-genic capabilities. This was seen across all plant groups [39]. While it is simple to start in vitro cultures of some species, such as tobacco and carrot, starting cultures of other species can be challenging or even impossible (cereals, grain legumes, bulbous plants). The majority of wild species, including medicinal plants and plants that produce phenols, are more challenging or exceedingly challenging to work with. This is especially true of medicinal plants—donor plants. The donor plant must be in good condition and not be in a dormant state; it must be in the early phases of its intensive growth. Pre-treatment of rhizomes and bulbs often entails exposure to either low or high temperatures for varying amounts of time [17].

5.2. Explant.

The type of explant employed may have a role in determining the organogenesis capacity and genetic stability of the clonal material. The explant's physiological age is another important factor to consider. The most appropriate material to use is immature organs and differentiated cells taken from stem tips, axillary buds, embryos, and other meristematic tissues. Nevertheless, despite the advances in cell and molecular biology, there are still limitations to the ease with which one can obtain information regarding the genetic, epigenetic, and physiological status of the explant. The empirical method is the one that is utilized most frequently when attempting to specify the chemical and physical stimuli that trigger cell totipotency.

5.3. Nutrient Media

Although over fifty different media formulations have been utilized for the in vitro development of plant tissue from a wide variety of species, the formulation published by Murashige and Skoog (MS medium) [31] is the most extensively used, with only minor modifications. [11], Huang and Murashige [14], Nicht and Nischt, etc., are only a few other well-known publications. In most cases, the nutritional medium will already include all of the macro and micro salts, vitamins, plant growth regulators, a carbohydrate, and other organic substances that the plant needs to thrive [35]. Growth regulators for plants. Plant growth regulators, including phytohormones, are necessary for the dedifferentiation, division, and redifferentiation of cells, ultimately creating callus tissue and organs. When it comes to development and morphogenesis in vitro, auxins and cytokinins play the most significant roles. However, the type of explants used and the genotype of the donor plant all have a role in determining which plant regulators are most effective, as well as the concentrations of those plant regulators in the nutritional medium. As a result, a large number of possible combinations might be devised, and the

most effective ones would be experimentally verified. All of this contributes to the challenges the experimental effort faces, which is centred on locating a happy medium between the elements that determine dependable in vitro growth. Cytokinins. To stimulate organogenesis and produce a dense bud set, the best cytokinins to use are either those found in nature (zeatin and kinetin) or those created in a lab (6-benzyl amino purine (benzyl adenine (BA, BAP), 6-(-dimethylallyl-amino)-purine (2iP), and thidiazuron). Possibly utilizing a different class of cytokinins. However, these are the most likely candidates (TDZ). Auxins. In addition to synthetic auxins like 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), picloram, etc., auxins can be derived from natural plant materials like indolyl-3-acetic acid (IAA), indole-3-butyric acid (IBA), and - naphthyl acetic acid (NAA). Auxins influence many plant growth and development aspects, from morphogenesis to cell differentiation. Depending on their molecular makeup and concentration, they can promote callus formation, root development or even prevent cell division. Gibberellins. About eighty chemical molecules fall under the umbrella term "gibberellins." Cells are encouraged to divide to grow and multiply in response to these molecules. In most cases, gibberellic acid is what's used (GA3) and added nutrients and vitamin pills. Commonly employed vitamins in vitro recipe creation include the B vitamins thiamine (B1), niacin (Vitamin B3, nicotinic acid, vitamin PP), and pyridoxine (Vitamin B6), which are associated with the capacity to control growth. It is possible that the use of certain nutritional supplements, such as yeast extract, coconut milk, maize extract, and others, might influence the maturation of buds and tissue. Optimum morphogenesis may be achieved if the optimal balance is discovered between the influences of genotype, explant, and growth regulators.

6. Rooting, Acclimatization, and Adaptation

It ought to be noted that the processes of root creation and adaptation each have their own unique prerequisites and that phylogenesis and adaptation do not always follow all instances of organogenesis, embryogenesis, or regeneration. These processes are dependent on the genotype and, in the majority of cases, the ecotype of the species [35], whilst the selection of the requisite culture conditions is done in an empirical manner. Root induction and formation are both helped by lowering the sucrose concentration from 2% to 3% to 1% to 0.5%. Acclimatization of the in vitro plants that have grown to maturity is an essential step in developing an effective micropropagation procedure. Another sensitive and challenging stage is when the plants adapt to their environment in the greenhouse, the field, or nature. Well-developed root systems may typically be regenerated in vitro under the appropriate circumstances.

On the other hand, they rapidly lose their turgor after they are planted in the soil. Their leaves became brown and shrivelled away. These plants experienced stress as a result of the adjustments in humidity and culture media that were made.

6.1. In vitro Cultured Conditions

In-vitro culture of plant cells and tissues requires a number of key conditions to be satisfied, including light, temperature, and air humidity. When it comes to morphogenetic processes, such as the creation of buds and shoots, the stimulation of root growth, and somatic embryogenesis, light is one of the most significant variables. The wavelength, intensity, and duration of light, as well as the length of the photoperiod, are critical factors in effective cultivation [31]. Maintaining a temperature of around $23\text{-}25^0$ C in the growing chambers or phytotron chamber is ideal. However, a higher temperature ($27\text{-}30^0$C) is needed for tropical species, while a lower temperature is needed for arctic plant cultures ($18\text{-}21^0$ C).

In vitro propagation procedures, often known as plant cloning, have been devised for several therapeutic plants [8], *Aloe vera* [47], *Angelica sinensis*, *Gentiana davidii* [(Satish *et al.,* 2013), *Chlorophytum borivilianum* (The establishment of a micropropagation system serves as a foundation for the preservation of the species, the protection of the gene pool, and the investigation of the key medicinal plants' vital constituents and compounds. Additionally, several methods are being developed for the formation of cell cultures with the purpose of manufacturing biologically active substances. These systems can potentially be utilized for the culture of plant cells at a large scale to acquire secondary metabolites. These techniques are dependable and provide the opportunity for a constant supply of raw materials to manufacture natural products [36].

The rate of acclimatization and adaption was found to vary greatly amongst species. Early on in the research, Pawlowska and Bach found that rooted plantlets of *Gentiana pneumonanthe* had a 65% chance of surviving in a greenhouse environment after being potted in soil and placed in containers. In addition, the plants were successfully grown outside in field circumstances after being planted. The number of flowers and stems on these in vitro regenerants was significantly higher than that of plants growing in their natural environments. After six weeks of culture, in vitro, plantlets of Gentiana punctata have been successfully acclimatized and transferred to soil. This was accomplished after the plantlets were grown in culture for six weeks.

Additionally, a substrate consisting of peat was employed effectively in order to root plantlets of *Gentiana dinarica*. It was found that combinations of vermiculite and turf were ideal for the acclimatization process of plants that had already formed roots and had been transplanted to pots in growth chambers. The HPLC analysis used to measure the number of secondary metabolites present in the clones showed that all of the acclimatized plants (one hundred percent) survived and stayed healthy. The samples were tested, and the results showed that they included gentiopicroside, organic acid, swertiamarin, and seaside. It was discovered that the predominant compound was the gentiopicro side.

Another morphogenetic process for the regeneration of plants is called somatic embryogenesis, and it is generally regarded as the most effective method for the regeneration of plants. In contrast to organogenesis, in which the buds and shoots do not always start from a single cell, a somatic embryo develops from a single cell. The improved genetic stability and parentage verification that results from this type of development are well worth the extra time and effort. This paves the way for the mass synthesis of artificial seeds and the wholesale replication of useful plants. Somatic embryogenesis, on the other hand, is trickier to achieve. In spite of this, it was successfully induced in a number of different Gentiana species, including *Gentiana lutea, Gentiana crassicaulis, Gentianacruciata], Gentiana pannonica, Gentiana tibetica, Gentiana pneumonanthe* and *G. kurroo* Royle, *Gentiana davidii* var. formosana (Hayata), and *Gentiana straminea*.

Another crucial component is the presence of plant growth regulators, which are needed to activate plant cell totipotence and lead to the development of a somatic embryo. While numerous cytokines and auxins stood out among the many studied plant growth regulators during organogenesis and shoot development in gentians, it was more challenging to identify the best ones during somatic embryogenesis. Several cytokines and auxins were shown to have a more important role in Gentiana organogenesis and shoot development.

Many different permutations of both naturally occurring phytohormones and manufactured phytoregulators were tested. As examples of auxins, we have -naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and 3,6-dichloro-o-anisic acid (dicamba), as examples of cytokinins, we have zeatin and 6-furfurylamonopurine (kn); however, indole acetic acid, a naturally occurring auxin, is not included. It indicates that more synthetic auxins than previously thought were employed in the studies.

Cell suspensions derived from the cotyledon and hypocotyl of the *Gentiana karroo* plant yielded protoplasts with extraordinarily high vitality, ranging from 88% to 96%. Three distinct culture methods and six different mediums were evaluated for their ability to produce healthy cultures and produce new plants from scratch. Agarose bead cultures grown in medium with 0.5 mg/l2,4-D and 1.0 mg/kinetin yielded the most successful plating results. The cotyledon and hypocotyl suspension yields from these cultures were 68.7 and 58.1 percent, respectively.

Another mechanism for plant regeneration was found when embryos were transplanted into MS medium diluted to half their initial strength. While flow cytometry showed increased DNA in one-third of the regenerants, this severely limits the use of isolated protoplasts in endangered species conservation and reproduction efforts. Therefore, research into what causes reliable plant regeneration has returned to the field of cell and tissue culture. This involved studying the correlation between photosynthetic activity and sucrose concentration in embryo culture media.

7. Genetic Transformation

The modification of genes paves the way for novel approaches to creating molecules with physiologically active properties. *Agrobacterium rhizogenes* is responsible for the growth of hairy roots, which leads to a rapid accumulation of biological material. *Agrobacterium rhizogenes* was used in the process of doing genetic engineering on *Rhodiola sachalinensis* [46]. The authors investigated the conditions that led to the high production of salidrosides, the primary compounds extracted from the roots of *Rhodiola sachalinensis*, by adding precursors (tyrosol, tyrosine, and phenylalanine) and elicitors (*Aspergillus niger*, Coriolus versicolor, and Ganoderma lucidum) to the medium. The optimal light intensity, pH, and nitrogen levels were sought to maximize salidroside synthesis. An elicitor concentration of 0.05 mg/l and a precursor concentration of 1 mmol/l were found to be optimal. Salidroside production was optimized at a scatter light intensity of 1000 lx, a pH range of 4.5 to 4.8, and a nitrogen content of 80 mmol/l (NH^{4+}: NO^{3-} =1:1). The authors conclude that Rhodiola's hairy roots can be used as a substitute material for the production of secondary metabolites with value in the pharmaceutical business.

8. Conclusion

The purpose of this analysis was to offer light on how plant biotechnologies may be used to save valuable plant species, such as medicinal plant species, that have become endangered or are on the verge of extinction due to human activity. The data and results that were presented in this chapter were intended to do just that. Growing important medicinal plants in controlled settings might be one strategy for addressing this worldwide issue and finding a solution. In order to accomplish this goal, "green" biotechnologies can be deployed in conjunction with the conventional practices employed in agricultural fields and nurseries. A significant number of researchers have come to the conclusion that plant biotechnology is essential for the propagation of rare and endangered medicinal plant populations as well as their preservation. Using in vitro methods that are kind to the environment makes it possible to grow an enormous number of identical plants, which can then be propagated, regenerated, and released back into the wild, therefore repairing and extending natural ecosystems.

On the other hand, if the important raw material could be produced in different ways, then the regions of the medicinal plants would be subjected to less risky exploitation. This would be a positive development. Because of this, vast volumes of biomass may be generated constantly and/or for a short period of time through the process of micropropagation of plants. Additionally, the manufacture of biologically active chemicals under controlled laboratory settings helps to lower the usage of natural resources, which in turn safeguards the species. The reality that in vitro propagation, cells, tissues, organs, and plantlet scans all produce metabolites unique to the full donor plant is of great importance when it comes to producing the required chemicals. The food, nutraceutical, pharmaceutical, and cosmetic sectors have all benefited

greatly from the revolutionary developments in the high-tech manufacture of valuable chemicals and physiologically active molecules. More advanced equipment and novel techniques that enable biotransformation and metabolic engineering have made these advancements possible. Murashige and Skoog medium (MS), N6-benzyl amino purine (BAP), indolyl-3-acetic acid (IAA), 6-(y,y)-dimethylallyl amino purine (2-IP), 2,4-dichlorophenoxyacetic acid (DCHA), naphthyl acetic acid (NAA), and thidiazuron (TDZ) are all abbreviations for various chemicals.

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