

**Mini Review**

Volume 4 Issue 2 – July 2017  
DOI: 10.19080/AIBM.2017.04.555631

Adv Biotech & Micro

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# Impediments in the Tissue Culture of High Altitude Medicinal Plants



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**Submission:** June 06, 2017; **Published:** July 17, 2017

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## Abstract

High altitude medicinal plants (HAMPs) are facing tremendous pressure of over-exploitation. Most of them have narrow distribution range, unique for their medicinal properties and sensitive to environmental perturbation. In addition, poor seed germination, low seed viability, lesser survival percentage and slow growth further puts pressure on the overall growth of HAMPs. In this context, tissue culture offers a sustainable way for conservation and sustainable utilization. The production and extraction of the active constituents through tissue culture approaches are increasingly growing. Large numbers of protocols are available for propagating and utilizing HAMPs through tissue culture, still the commercial exploitation is meager. The present article highlights various impediments that a researcher might face during the tissue culture of HAMPs. The sustainable utilization of these plants through tissue culture needs collaboration with the various experts from the field of life, engineering and management sciences so that suitable conditions can be optimized for their commercialization.

**Keywords:** Active constituents; Conservation; High altitude medicinal plants (HAMPs); *In vitro* propagation; Optimization

**Abbreviations:** WHO: World Health Organization; HAMPs: High Altitude Medicinal Plants

## Introduction

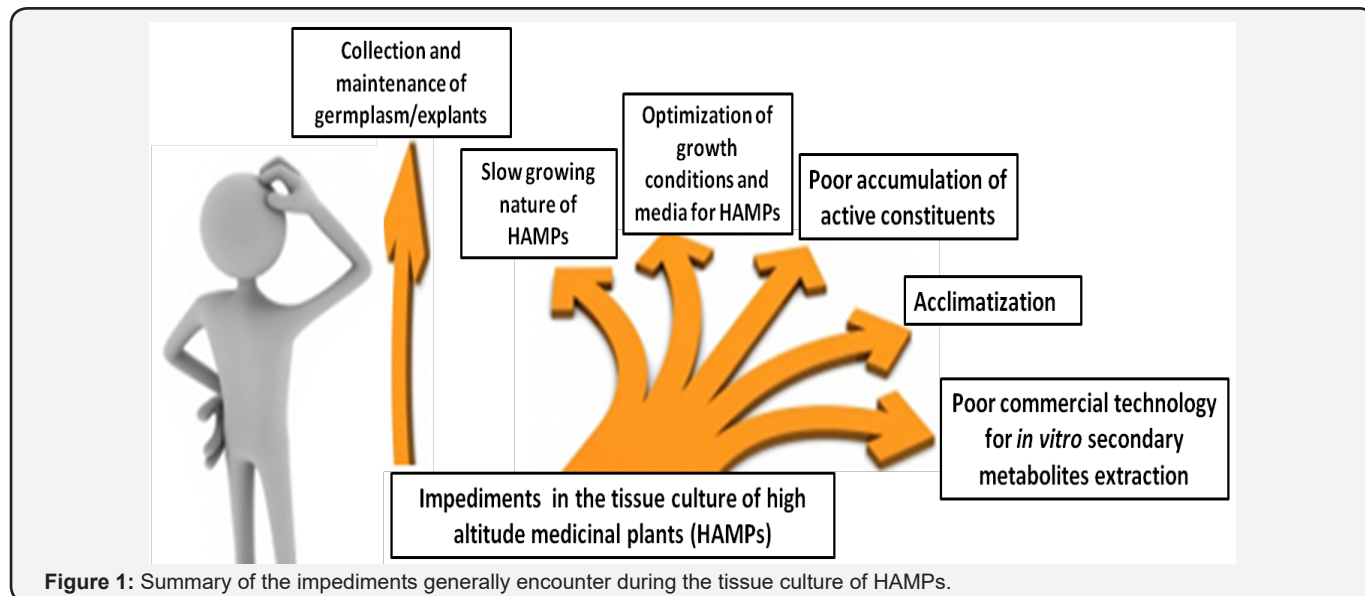
The world health organization (WHO) estimated that more than 80% population of the developing countries depends on the medicinal plants for primary health care [1]. About two third of the medicinal plants in use are collected from wild [2]. As such, the demand of the medicinal plant based raw drugs is increasing at the rate of 15 to 25% annually and may reach more than US\$ 5 trillion in 2050 [3]. The HAMPs are being over-exploited and threatening their existence particularly due to the increasing herbal medicine demand, deforestation, overgrazing, rapid agricultural expansion, habitat destruction, destructive harvesting and by the climate change. It has been reported that nearly 4000 to 10000 medicinal plants might now be endangered [2]. The major target of plant tissue culture includes sustainability, food security, and the production of novel biomaterial [4]. Advancement in plant tissue culture provides new means for conservation and rapidly propagating the valuable, rare and endangered medicinal plants [5]. In addition, the plant tissue culture offers integrated approaches for the mass production of bioactive constituents via callus or

suspension cultures. Plant tissue culture thus promises efficient and reliable production system for phyto chemicals as well plants as a whole [6].

High altitude medicinal plants (HAMPs) are the choice for pharmaceutical industries as they hold greater possibilities of having novel molecules and even larger quantities of active compounds due to their exposure to extreme climatic and geophysical conditions [7,8]. The indiscriminate exploitation coupled with unregulated harvesting and lack of attention to strategic cultivation practices have led to the endangered or threatened status of several HAMPs [9]. Considering to this, several researchers are working for the development of *in vitro* propagation and production of active constituents from these species. Several HAMPs are successfully propagated and used as the source of active constituents by tissue culture methods [5,10-20]. Unfortunately, most of the research remains up to the laboratory and commercial propagation of these plants is meager. The researchers face several impediments

in development of the successful and replicable tissue culture protocols for such species. In the present article we tried to

summarize the impediments that a researcher might face during the tissue culture of HAMPs (Figure 1).



**Figure 1:** Summary of the impediments generally encountered during the tissue culture of HAMPs.

### Collection and maintenance of germ plasm/explants

High altitude regions are characterized by extreme weather conditions such as cold and arid climate, scanty rainfall, heavy snow fall, high wind velocity, avalanches, landslides etc. The angle of slope is generally high. Environmental conditions in alpine areas change sharply within pockets [21]. The process of initiation of plant material into culture usually occurs in the laboratory after collection from the field [22]. The collection of the plant material from the high altitude region is not an easy task and it needs specialized collection techniques. Locations of the availability are generally remote. The transport facilities are very poor, and the plant collectors might need to walk several kilometers to reach the exact locations. Further, there is either lack of geo-coordinated data regarding locations (as high altitude regions are less explored) or sometimes when the reports available, one does not find them at all on reported locations due to exploitation and destruction of habitats by anthropogenic and natural factors (flash flood and landslides). Thus, eventually the plant collector has to look for alternate locations which might accommodate limited number of plants. Under such conditions the replicates of experiments have to be reduced accordingly. The researcher has to carry the plants to the laboratory/arboretum from a far flung region. Transporting plant/explants to laboratory/arboretum is yet another tedious job. The maintenance of these plants outside their natural habitat (at low altitude) is not easy and their survival is usually very low. Thus, it is difficult to maintain the continuous supply of explants for the running experiments. It is also evidenced that the limited amount of plant material available from rare species poses major challenges in the application of *in vitro* methods to endangered species [22].

### Slow growing nature of HAMPs

Alpine plants are generally slow growing. Seeds of alpine plants may remain dormant for long period at low temperature since they require temperature well above the freezing for germination [23]. Threatened alpine plant species are often slow to reach maturity (7-8 years) and to produce the tradable component [24]. It is also known that the seedling establishment in alpine plants is rare and very slow; it may take several years before safe establishment. HAMPs often show similar growth performance in the *in-vitro* conditions. HAMPs cells generally have low biomass yields in callus and suspension cultures as compared to the other plants. The slow growth of HAMPs in *in-vitro* conditions incorporates the risk of contamination by fast growing bacteria/fungi. Contamination is of great concern for the large scale propagation and cultivation of HAMPs.

### Growth conditions and media for HAMPs

The HAMPs prefer highly specific habitat [25]. Most of the alpine plants grow in association with other plants as these associations normally result in increased biomass, growth and reproduction of sub-alpine and alpine species [26]. The cultivation of HAMPs generally requires a thorough knowledge on the biology, otherwise *ex situ* replication of these conditions is tedious. As such, there is lack of published methods for *in vitro* propagation of medicinal plants and this makes the choice and development of the initiation culture medium arbitrary [27]. Even if the cultures are initiated, the growth and multiplication of *in vitro* cultures can remain problematic requiring specific novel treatments [22]. The growth conditions of large number of medicinal plants are yet to be optimized [28]. There are number of chemical and physical factors like media components, phyto hormones, pH, temperature, aeration, agitation, light, etc. that

are to be optimized for the better growth of the plants in *in vitro* conditions. This needs long time and huge efforts which may hinder the interest of the researchers and one of the major impediments in the large scale quality plant production.

### Accumulation of active constituents

The accumulation of active constituents varied greatly within and among the species, habitat and altitude [29]. These observations are equally true in *in vitro* conditions where variations in the physical and chemical environment may lead to the variation in accumulation of active constituents. HAMPs generally accumulate more bioactive constituents in natural conditions in response to extreme climatic and geophysical conditions [8]. Although, reports are available for the accumulation of more bioactive constituents in *in vitro* cultures and non native habitat as compared to natural habitat [18,30] but, this require a perfect optimization of plant growth regulators and media type which is being used. In most cases, plant cells often produce reduced quantities of active constituents *in vitro* and their qualitative features may also be changed. Better results can be obtained only after manipulating the environmental parameters and medium, selecting high yielding cell clones, precursors feeding and elicitation [31]. Obtaining these results is very laborious, costly and time consuming affair, however, at the same time if it get optimized and can stable the production will help in reducing pressure on the natural sites.

### Acclimatization

The use of *in vitro* propagated HAMPs for reintroduction is one of the critical step; however, it relies on the successful methods for acclimatization. Acclimatization of the tissue culture raised plants has never been an easy task. A substantial number of tissue culture propagated plants do not survive from *in vitro* conditions to green house or field environment [32]. The condition becomes more adverse for HAMPs as the natural fields are generally far apart and the greenhouse or non-native field may have changed humidity, light level and other environmental conditions as compared to native environment of the species. The transplantation continues the major bottleneck in the conservation of HAMPs as most of them require specific protocols for the successful acclimatization.

### Commercial consideration

Despite the fact that, lots of protocols have been developed for the *in vitro* propagation of HAMPs and the active constituent extraction from *in vitro* cultures has also been done successfully very few workers focused on the cost-benefit analysis of these protocol [4]. Due to this the commercial exploitation of these protocols are still lacking [33]. It is often observed that most of the pharmaceutical or medicinal plants based industries are still collecting and extracting the active constituents from the wild plants as there is no commercial technology for *in vitro* secondary metabolites extraction.

### Conclusion

In spite of several impediments in the propagation of the HAMPs, the importance of tissue culture in the conservation and production of secondary metabolites from *in vitro* raised culture are gaining popularity. A plenty of literature is available wherein researchers have significantly overcome with field and laboratory problems and implemented new ideas and innovations [5,10-20]. In addition, methods of seed banking, development of living collections, cryopreservation and molecular genetics may provide additional backup for propagation and conservation of HAMPs. Greater progress can be expected if the plant culturists are given more opportunities to closely collaborate with the chemists, biochemist, biotechnologist, molecular biologist, geneticists and engineers. The commercialization of tissue culture techniques for HAMPs needs more time and patient. Researchers working in this area need motivation and facilitation by special recognition of their contribution.

### Conflict of Interest

Authors declare no conflict of interest.

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DOI: [10.19080/AIBM.2017.04.555631](https://doi.org/10.19080/AIBM.2017.04.555631)

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