

Review

***Aconitum balfourii* Stapf: A rare medicinal herb from Himalayan Alpine**

Eti Sharma and A. K. Gaur*

Department of Molecular Biology and Genetic Engineering, College of Basic Science and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar-263145, Uttarakhand, India.

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***Aconitum* is a genus of flowering plant belonging to buttercup family (Ranunculaceae). Globally, there are over 300 species of *Aconitum*. In India, the genus is represented by about 24 species mainly distributed in subalpine and alpine zones of Himalayas. Out of these species, *Aconitum balfourii* Stapf, known as Mitha and Vatsnabh, is a significant species of this genus. It is widespread in Kumaon and Garhwal Himalayas on shady slopes from 3000 to 4200 m altitudes. Tuberos roots of *A. balfourii* Stapf are rich sources of pseudoaconitine (0.4 to 0.5%) and aconite alkaloids. The value of aconite as a medicine has been fully recognized in modern times, and it now ranks as one of the most useful drugs, particularly in Homeopathy, Ayurveda, and Unani systems of medicine. Due to overexploitation, *A. balfourii* Stapf is facing severe threat, and the plant has been listed among 37 Himalayan medicinal herbs under priority for *in situ* and *ex situ* conservation. This review mainly discussed several aspects, such as: distribution, cultivation, morphological characteristics, basis of origin, and conservation of this important species of the genus *Aconitum*.**

Key words: *Aconitum*, conservation, evolution, propagation, pseudoaconitine.

INTRODUCTION

Plants represent an immense biodiversity with more than 250,000 known species existing within the plant kingdom (Phillipson, 2003). About 50,000 species are used as raw material for the synthesis of herbal medicines by the pharmaceutical companies worldwide, which is about 13% of all flowering plants (Schippman et al., 2002; Maiti, 2005). Approximately, 8000 species of medicinal plants are used as different systems of medicines in India (Planning Commission, 2000). India is blessed with huge biodiversity due to different climatic zones, in which numerous medicinal plants were reported. The Indian state of Uttarakhand, located in Himalayan region, is richly gifted with a large variety of plant species, many of which have medicinal properties. Medicinal plants play an important role in the lives of people in Uttarakhand by providing basic health care and employment to the farmers (Alam and Kop, 2005). *Aconitum* species belong

to the family of Ranunculaceae, which is chiefly found in Uttarakhand with other medicinal plants. *Aconitum* genus is widely distributed in the Himalaya region. About 300 species of *Aconitum* is found in the world of which 24 species are found in great Himalaya of India. *Aconitum balfourii* Stapf is an important highly prized herb of this genus. It is endemic to the alpine and subalpine belts of Indian Himalayan region. It grows between 3000 to 4300 m asl (Chopra et al., 1984; Samant et al., 1998). The plant is a perennial herb with fleshy, spindle-shape root containing alkaloids pseudoaconitine, a highly toxic alkaloid as a principal component, and aconitine, benzylaconitine, picroaconitine, and haemonepellene in traces (Anonymous, 1985). The pharmaceutically significant compound present in the roots of *Aconitum* is a type of diterpenoid alkaloids (Sultankhodzhaev and Nishnov, 1995) which form the ingredient of Ayurvedic and Homeopathic system of traditional medicine. In the past decades due to destructive anthropogenic activities and poor rate of natural regeneration of *A. balfourii* Stapf, this high value of plant species has acquired threatened status.

*Corresponding author. E-mail: anilgaur123@rediffmail.com.
Tel: + 919412120798. Fax: 05944233473.

Table 1. Distribution of *A. balfourii* Stapf. at different locations of Garhwal Himalaya.

Site	Altitude (m)	Frequency (%)
Dayara	3000 - 3300	60
Hari Ki Dun	3100	50
Kedarnath	3450 - 3650	45
Madhyamaheswar	4300	40
Panwalikantha	3300 - 3400	45
Tungnath	3500 -3600	60
Valley of Flower	3000	30

ORIGIN

Several taxonomic studies were carried out to understand the highly difficult taxonomy of the genus *Aconitum*. The taxonomy is tough to recognize because aconites are morphologically extreme variables (Kadota, 1987; Yang, 1990). Recently, several attempts have been made to understand the molecular phylogeny and phylogeography of *Aconitum* by sequencing the internal transcribed spacer (ITS) region of chromosomal deoxyribonucleic acid (DNA) or non-coding region of chloroplast DNA (cpDNA). In one study, they took 51 species and one variety from Eastern Asia, North America, and Europe. On the basis of ITS data and the unique morphological characteristics of the species, genus *Aconitum* was classified into two sections: *Sinaconitum* and *Aconitum*. In their study, they did not take any Western Himalayan species of *Aconitum*, but on the basis of morphological similarities they considered *Aconitum heterophyllum* as a western Himalayan species in section *Sinaconitum* (Kita and Ito, 2000; Luo et al., 2005).

There is limited information about the evolution of *Aconitum* in India, because studies are lacking the evolutionary aspects of *Aconitum* in India. Many researchers have studied the evolutionary aspects of Asian and European *Aconitum* and tried to correlate the evolutionary relationship between them. For that, they have studied different species from different biogeographical zones. In evolutionary study, reticulate evolution of high alpine *Aconitum* was studied on the basis of chromosomal and molecular polymerase chain reaction-inter-simple sequence repeat (PCR-ISSR) pattern and they have studied the species from Eastern Sudetes and Western Carpathian of central Europe and concluded that in Central Europe, Sudetic mountains might be the oldest source of genetic diversity of *Aconitum* and old homotetraploid form of *Aconitum* was persisted in this region. On the basis of their study and previous studies, they suggested some correlation between Asian and European *Aconitum* on the basis of phylogeny in which both species, despite the huge geographical distance, shared the same cluster and are considered as sister in the phylogenetic tree (Mitka et al., 2007).

GEOGRAPHICAL DISTRIBUTION

The genus *Aconitum* comprises of 300 species in the world with major centers of diversity in the mountains of East and South-East Asia and Central Europe (Kadota, 1987). A small group is also found in Western North America and Eastern United States of America. In Himalayan regions, it is distributed in Pakistan, India, Nepal, Bhutan, and South Tibet, where *Aconitum* were used in local and traditional system as medicine (Shah, 2005). In India, the genus is represented by about 24 species that is mainly distributed in sub alpine and alpine zones of Himalayas from Kashmir to Uttarakhand and extending it to the hills of Assam. *Aconitum* is usually found in wet alpine zone, however, in Kashmir Himalayas, these species were reported in temperate zone. Out of 300 species, a total of 33 species are found in great Himalaya (Chaudhary and Rao, 1998). *A. balfourii* Stapf is prevalent to Garhwal and Kumaon regions; it is also found in Nepal. The distribution of *A. balfourii* Stapf in different zones of Garhwal region is presented in (Table 1). It is generally found in the valley of flowers, Kedarnath, Tungnath, Madhyamaheshwar, and Panwalikantha on shady slopes between 3000 to 4200 m altitudes (Chopra et al., 1984).

CURRENT STATUS

Overexploitation and habitat destructions are two major causes of threat categories of this species. Population density and degree of consistency (occurrence) was used to allocate the status of any species. The population study of three aconites, *A. balfourii*, *A. heterophyllum* and *A. violaceum*, was carried out in Garhwal region. In this study, the authors considered many regions in Garhwal and revealed that *A. balfourii* has the highest frequency (70%) and density at 3200 m (near timberline) in Dayara and the minimum (30%) in the valley of the flower. The degree of consistency of *A. balfourii* was frequently found at most sites and seldom in one or two pockets. They recommended that it might be due to its specific habitat requirement or due to continuous removal of plants for the medicinal uses. On the basis of their study, they have assigned *A. balfourii* and other two aconites as endangered

Table 2. Taxonomic position of *A. balfourii* Stapf.

Kingdom	<i>Plantae</i> : Plants
Subkingdom	<i>Tracheobionta</i> : Vascular plants
Superdivision	<i>Spermatophyta</i> : Seed plants
Division	<i>Magnoliophyta</i> : Flowering plants
Class	<i>Magnoliopsida</i> : Dicotyledons
Subclass	<i>Magnoliidae</i>
Order	<i>Ranunculales</i>
Family	<i>Ranunculaceae</i> : Buttercup family
Genus	<i>Aconitum</i> L.: Monkshood
Species	<i>balfourii</i> Stapf. : Vatsanabha, Mitha Vish

species (Nautiyal et al., 2002). According to International Union for Conservation of Nature and Natural Resources (IUCN), *A. balfourii* is vulnerable and its regional status in Uttarakhand is also vulnerable (Bisht and Badoni, 2009). In a recent study on Indian Himalayana region, threat categorisation of the floristic diversity was undertaken based on conservation priority index. They considered a total of 637 species of vascular plants, 10 species were categorized as critically endangered, 15 species as endangered and 31 species as vulnerable. Their study considered *A. violaceum* in near threatened category and its status is vulnerable, whereas *A. heterophyllum* was considered as critically endangered and status is endangered in Himanchal Pradesh as well as globally. They have suggested that it is a consequence of over exploitation and habitat destruction (Rana et al., 2010). The taxonomic position of *A. balfourii* is represented in Table 2.

CLASSIFICATION ON THE BASIS OF TYPE OF ACONITINE

Another type of classification which is based on the type of aconitine present in a species, *Aconitum* species which have found in Uttarakhand region of India are classified into two classes: poisonous and non-poisonous *Aconitum*. In non-poisonous species include *A. heterophyllum*, *A. laeve* and *A. rotundifolium* and in poisonous ten species have been included these are *A. chasmanthum*, *A. ferox*, *A. deinorrhizum*, *A. falconeri*, *A. balfourii*, *A. moschatum*, *A. violaceum*, *A. spicatum*, *A. bisma* and *A. laciniatum* (Shah, 2005).

Genome base, chromosome numbers, and ploidy levels

A literature survey of chromosome number counts was made for the tribe *Delphinieae*, which includes the genera *Aconitum*, *Delphinium*, *Consolida*, and *Aconitella*. In this survey, 1097 reports were presented corresponding to

327 species, representing about 40% of the total species number of the tribe. The basic number is universally $x = 8$ and ploidy levels found are $2x$, $3x$, $4x$, $5x$, $6x$, and $8x$. Polyploidy is more frequent in perennial taxa (*Aconitum* and *Delphinium*). Genus *Aconitum* displays the larger diversity of ploidy levels within the tribe; however, the most frequent level is tetraploidy (79% of polyploidy counts). Some studies proposed that sometimes the chromosome number $2n = 24$ was noted and they suggested that it might be due to crossing between a diploid species and a tetraploid species (Zielinski, 1982; Tomasz and Mitka, 2009).

A. balfourii is a tetraploid species ($2n = 32$) which was reported in cytological examination study of micro propagated plants of *A. balfuorii*. Number of chromosomes ($2n = 32$) identical to that of seedling plants is stable even after passing through a prolonged period of culture (ten cycles of shoot multiplication) followed by 12 months under greenhouse conditions (Pandey et al., 2004).

Botanical description

A. balfourii Stapf is an erect, glabrous shrub that is more than 1.5 m in height. Root tubers are 7 to 12 cm long and are extraordinarily heavy. Flowers in raceme with five sepals, petaloid and free upper one petals form a large erect hood. Upper two petals forming nectar secreting spur, lower two petals are small. Roots are tuberous and fusiform. Stem is simple or branched, about 1.2 m high, dull purplish-brown. Lower leaves are long stalked and upper ones are short stalked (Gaur, 1999; Stapf, 1905).

Floral characteristics

The flowers are hermaphrodite and are pollinated by bees. Inflorescence is about 29 cm long containing many flowered racemes with yellowish tomentum. There are five blue sepals with pubescent, uppermost helmet-shaped.

Petals glabrous, upper two hooded enclosed in the helmet. Stamens are many; filaments hispidulous and pollens are 3-colpate. Carpals are 3 to 5, yellowish, tomentose. Follicles are 2 to 5, sessile, silky pubescent. Seeds are broadly winged along raphe. The favorable time for flowering and fruiting in *A. balfourii* is September to November (Gaur, 1999).

Seed germination

Seeds of *A. balfourii* Stapf are dormant in nature. Under natural conditions, seed germination and seedling establishment in *A. balfourii* Stapf is very difficult. Seeds are best sown as soon as it is ripe in a cold frame. The seed can be stratified and sown in spring but then will be slow to germinate. Seed sowing may be done from October to November or March to April in mist chambers and shade houses. Its division is best done in spring but it can also be done in autumn. Some treatments such as treatment of the seeds with gibberellic acid and sun drying of seeds before sowing, followed by mulching are recommended for better germination of seeds in *A. balfourii*. Some chemical treatments such as the treatment of thiourea and ammonium nitrate also helped to improve the germination of seeds of *A. balfourii* and *A. heterophyllum* (Pandey et al., 2000). The effect of pre sowing of seeds in different chemicals and temperature was studied in *A. heterophyllum* and was found that low temperature and 0.5 mg/lit IAA concentration was suitable for seed germination (Srivastava et al., 2011).

Besides the chemical treatment, some physiological factors such as light and temperature also affect the germination behavior in *Aconitum*. A recent study on *A. deinorrhizum* Stapf revealed that seeds exposed to continuous light conditions with 20°C temperature gave better response in terms of seed germination. Seed germination percentage was found higher (76.67) as compared to continuous dark condition (63.60) at the same temperature. In their study, they recommended that 20°C temperature is most suitable for seed germination in this species on the basis of peak value, germination value and germination energy (Sood and Thakur, 2011).

Reproductive biology

Variation in the flowering potential, seed characteristics and seed germination behavior in the populations of *A. balfourii* Stapf were studied and it was observed that alpine populations of Dayara and Valley of Flowers produce more flowers per inflorescence, and the subalpine populations produce less number of flowers per inflorescence. The length of floral axis was recorded higher in alpine populations than in subalpine populations. The alpine populations were recorded with more flowers per plant compared to subalpine population. It

was suggested that alpine populations of the *A. balfourii* Stapf adopt mass flowering strategies (Kuniyal et al., 2003).

Minor variations were also noted in different populations in fruit weight, number of seeds per fruit and seed length and width. Seed germination studies revealed that alpine populations germinate quickly than that of subalpine populations under photoperiod conditions. It was observed that shade is a detrimental factor for flowering and seed production in wild habitats (Nautiyal et al., 2009). Furthermore, domestication of species in the hot house showed optimal flowering and seed production. Variation in time of anther dehiscence and stigma receptivity indicate protandry form of dichogamy in individual flower to present the potential for cross pollination. Protandry in particular is viewed as an anti-selfing mechanism because it provides opportunities for the receipt of outcross pollen before self pollen is shed and is more common in self compatible than self incompatible taxa (Lloyd and Webb, 1986; Berlin, 1993). Floral biology and breeding system of *A. balfourii* Stapf indicate the species reproductive potential for cross pollination, which would limit the production of selfed seeds and as such is likely to maintain sustainable level of heterozygosity among wild populations (Nautiyal et al., 2009).

Propagation material, cultivation details and varieties

Propagation is generally done through seeds and tuber segments. Stem cuttings have also been found to be successful in multiplication at higher altitudes. There is little information of cultivation of *A. balfourii* Stapf. Area above 2200 m altitude is suitable for the cultivation of Indian aconite. Sandy loam and slightly acidic (pH 5.1 to 5.5), rich in humus, are suitable of cultivation of this crop. Partially shaded areas, thick soil and moist conditions provide a healthy environment for the plants. It also prefers a calcareous soil.

Propagation study through tuber segments was carried out in alpine region in *Aconitum atrox*. It was attempted at higher altitude (Rawat et al., 1992). Similarly, the same study was attempted but at lower altitude and in their study, they successfully propagated the plant by treating tube segments with a combination of GA₃, IBA, and Kinetin, a combination of any two of them or the use of any one of them for 48 h at room temperature. The study was helpful to overcome constraints in seed germination and seedling establishment (Kuniyal et al., 2006).

Members of this genus seem to be immune to the predations of rabbits and deer; it is a greedy plant, inhibiting the growth of nearby species, especially legumes. No variety of this plant has been identified. The strains collected from timberline population show better survival and good response to vegetative growth and yield, when cultivated at comparatively lower altitude.

Disease and pest control

There is no observation of any serious disease affecting the plant, although insect may harm the flower. No chemical pesticide or insecticides are applied to the crop.

Chemical constituents and biological importance

Tubers of *A. balfourii* Stapf mainly contain a crystalline toxic alkaloid called pseudoaconitine (0.4 to 0.5%) and aconitine in small amount (Figure 1). Three new norditerpenoid alkaloids 8-O-methylveratroylpseudoaconine, veratroylbikhaconine, and balfourine have been isolated from *A. balfourii* Stapf together with eight known alkaloids such as: pseudoaconitine, veratroylpseudoaconine, indaconitine, ludaconitine, 8-deacetylyunaconitine, bikhaconitine, neoline, and chasmanine. From the aerial parts of *A. balfourii* Stapf are nine norditerpenoid alkaloids: condelphine, bullatine, neoline, isotalatizine, 1-O-methyldeiphisine, pseudoaconitine, yunaconitine, bikhaconitine, and indaconitine were isolated (Khetwal, 2007). Pseudoaconitine Figure 2 is a diterpene alkaloid, with the structural formula $C_{36}H_{51}NO_{12}$. The crystal melts at 202°C and is moderately soluble in water, but more so in alcohol. This shows that it is a lipophilic substance. When heated in the dry state, it undergoes pseudoaconitine pyrolysis and formed pyropseudoaconitine $C_{34}H_{47}O_{10}N$ (Tsuda and Marion, 1963).

Several diterpenoid alkaloids isolation and detection through HPLC protocols from the genus *Aconitum* have been standardized (Hikino et al., 1983; Jiang et al., 2005; Wang et al., 2006). The alkaloids present in this herb are toxic, which can easily turn into less toxic alkaloids by heating or alkaline treatment through deacetylation, debenzoylation or oxidation. This process is known as mitigation or detoxification. After mitigation, the alkaloids are used in Ayurvedic and Unani medicines. Alkaloids isolated from this genus exhibited anti-inflammatory, antinociceptive, hypotensive, bradycardic, analgetic, and cardiogenic activities (Ameri, 1998). The roots of *Bachnak* are diaphoretic, diuretic, febrifuge, anti-inflammatory, antirheumatic, antipyretic, and vermifuge. It is used in all types of pains and inflammations. In large doses, it acts as powerful sedative, narcotic and poison. The crude methanolic extracts of *Aconitum* species possess pharmacological activities such as antifungal, antibacterial, and insecticidal properties (Anwar et al., 2003).

REASONS FOR THE THREATENED STATUS OF *A. balfourii*

In the last three decades, the aconites of Indian Himalayas are mercilessly exploited. The consequence is

that many species of this genus was listed as an endangered taxon (Shah, 1983). There are several reasons behind the endangered status of *Aconitum*. Tuberos roots of *A. balfourii* Stapf are rich resource of the important aconite and pseudoaconitine alkaloids. The extract is used in different system of medicines, so the commercial demand is very high. To fulfill the demand of excessive illegal collection and sale of *Aconitum* by farmers was continuously carried out. Other reasons are the low germination percentage and the cultivation of *Aconitum* species are done in a very small scale because of low availability of land for cultivation of medicinal plants (Srivastava et al., 2010). In addition, under natural conditions, seed germination and seedling establishment in *A. balfourii* is very rare. Growing harvestable raw material from seeds requires a lengthy cultivation cycle of 5 to 7 years (Rawat et al., 1987). Moreover, destruction of natural habitat and the earlier mentioned reasons are collectively responsible for its endangered status.

Conservation efforts

There are two methods (*in situ* and *ex situ*) for the conservation of medicinal plants. The best method of conservation is for medicinal plants to grow and evolve in wild and their natural habitats. However, *in situ* conservation is achieved by setting nature reserves and national parks. Plants should also be conserved *ex situ* that is, to grow outside their habitat in controlled environment. Its advantage is that it is usually easier to supply plant material for propagation, for research and for educational purposes than from *in situ* reserves. The disadvantages of *ex situ* conservation are that the plants conserved *ex situ* may represent a narrower range of genetic variation than that which occurs in the wild and continuous human care is needed. For this reason, *ex situ* conservation must not replace, but should complement *in situ* conservation (Batugal et al., 2004; Senwal et al., 2007).

The first effort of conservation of medicinal plants in Uttarakhand by the Government was taken in 1985 by constituting a committee of experts by the Forest Department of Government of Uttar Pradesh. The committee banned the collection and marketing of medicinal species from Uttaranchal for a period of four years, vides State Government Order no. 535/1-9-20 dated January 1986. Among them, *A. heterophyllum*, *A. balfourii*, *A. deinorrhizum*, and *A. falconeri* were included (Shah, 2005).

Different studies have been carried out on the aspects of conservation of *A. balfourii* by means of micropropagation. In the area of micropropagation, efficient protocol for regeneration of *A. balfourii* and *A. heterophyllum* were reported (Bist et al., 2011; Pandey et al., 2004; Jabeen et al., 2006). The study of population genetic diversity is another method that provides useful



Figure 1. Tuber of *Aconitum balfourii*.

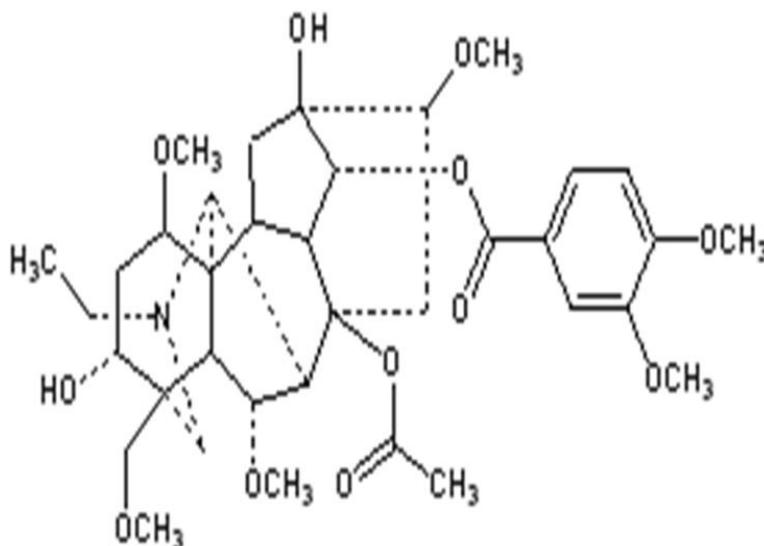


Figure 2. Chemical structure of Pseudoaconitine.

information for biological conservation. Four populations of *A. balfourii* Stapf were studied through polypeptide pattern and isoenzyme markers to locate the genetic diversity. They studied three enzyme systems such as: esterase, superoxide dismutase, and catalase. On the basis of isoenzyme variations and banding pattern, they have identified seven polymorphic loci in four populations (Pathak et al., 2011).

Somatic embryogenesis is an additional method for

conservation of threatened species of medicinal plants. It was tried in *A. heterophyllum*. Somatic embryos were obtained from callus by using 6-benzylaminopurine (BAP) (1 mg/L) and naphthaleneacetic acid (NAA) (0.1 mg/L). They successfully regenerated plantlet by transferring embryos in 1/4 strength MS nutrient with indole-3-butyric acid (IBA, 1 mg/L) (Giri et al., 1993). High polysaccharides and polyphenolics hinder good quality of genomic DNA isolation from *Aconitum*, therefore, DNA

isolation protocols was standardized for the genetic diversity analysis and other molecular biology experiments (Hatwal et al., 2011; Srivastava et al., 2010b). All these efforts are included in *ex situ* conservation. Conventional seed storage is an efficient method of *ex situ* conservation but the seed dormancy of *Aconitum* species limits this approach. Therefore, *in vitro* propagation is a more suitable way for conservation of *Aconitum* species (Srivastava et al., 2010a).

Efforts to enhance aconitine production

The alkaloids present in the genus *Aconitum* which have medicinal properties is the main reason for overexploitation. Therefore, several biotechnological tools was tried to enhance its production *in vitro* as well as *in vivo*. Hairy root culture established by transformation with *Agrobacterium rhizogenes* is regarded as an advantageous resource of useful compounds because of the rapid growth in culture media without phytohormones and relatively high productivity of secondary metabolites compared to undifferentiated calli or cell suspension, or in some case roots of mother plants. A method for the production of hairy roots of *A. heterophyllum* wall was reported in which they successfully transformed embryonic cell culture of *A. heterophyllum* wall by using *A. rhizogenes* strains such as LBA 9402, LBA 9360, and A4 for the induction of hairy roots. It was found that total alkaloid (aconite) content of transformed roots was 2.96%, which was 3.75 times higher compared to 0.79% in the non transformed roots (Giri et al., 1997). Plant suspension culture for optimization of aconitine production in *Aconitum napellus* cultures showed that many factors like sucrose concentration, elicitor like salicylic acid, and yeast extract affect the production of aconitine *in vitro* cultures (Huwang et al., 2004). Hydroponics (from the Greek words *hydro* water and *ponos* labor) is a method of growing plants using mineral nutrient solutions, in water, without soil. *In vitro* regenerated plantlets of *A. balfourii* were used for hydroponics cultures and proved to be beneficial for increasing root strength as well as aconitine content. The maximum percentage of aconitine (0.024%) was found in hydroponics cultivated plants followed by plants collected from Tungnath followed by (0.015%). Tissue culture derived from plants has minimum percentage of aconitine (0.0123) (Pathak, 2010).

CONCLUSION AND FUTURE DIRECTIONS

Genus *Aconitum* is well acknowledged for its benefits in different systems of medicines, and they play an important role in the field of safer herbal medicines, so the strain on their natural habitat has increased. Many species belong to this genus, one of which is *A. balfourii* Stapf. The plant needs some more attention towards the

researchers. There are several reasons for paying attention to this significant species, one of which is as a result of the fact that it contains a diterpenoid alkaloid pseudoaconitine in its tubers, which is 1.5 times more potent than aconitine. Due to the fact that it contains higher potency, it can work better in a pharmaceutical industry to fulfill the demand of aconitine in various systems of medicines. Moreover, *A. balfourii* faces a severe threat due to overexploitation, and only little work has been done so far on it. So, there is a need to target certain key areas including the development of appropriate structures for cryopreserved gene banks, the use of *in vitro* methods for the safe transfer of disease free germplasm to save *A. balfourii* from extinction.

The low level of alkaloid content, present in wild species, which is not able to satisfy the demands of pharmaceutical industries, is another problem which has to be conquered. Many biotechnological 'applications', such as plant cell suspension cultures and genetic transformations through *A. rhizogenes* for the production of hairy roots must be considered with a long-term perspective for *in vitro* production and enhancement of its active components. The studies are lacking in the area of pathway identification of aconitine biogenesis. It is beneficial to find out the key genes and enzymes that regulate the biogenesis of aconitine, which help in pathway engineering, and which is a useful tool in biotechnology to enhance the *in vitro* production of pharmaceutically important diterpenoid alkaloid. Hence, it is necessary to take a step forward for the conservation of these valuable aconites and all organizations have to participate in the conservation programmes.

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