



# Genetic stability and phytochemical profiling of the in vitro regenerated plants of *Angelica glauca* Edgew.: an endangered medicinal plant of Himalaya

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## Abstract

The present study describes the first successful report on in vitro propagation through direct organogenesis for multiple shoot induction of *Angelica glauca*. Rhizomes were used as explant, and maximum shoot multiplication was observed on MS medium supplemented with 6-Benzylaminopurine 8.0 µM and Indole-3-acetic acid 0.1 µM. Roots were observed within 14 days in the MS medium enriched with 0.5 µM IAA and 0.1 µM Naphthalene acetic acid (NAA) with an average production of 4.2 roots per shoot. Rooted plantlets were successfully hardened under greenhouse conditions and subsequently established in field, with a recorded survival rate of 72% after 45 days. The total phenolic content showed significant difference ( $p < 0.05$ ) between in vitro raised plants (5.87 mM AAE/ g DW) and control (2.36 mM AAE/ g DW). Antioxidant activity, calculated through two in vitro assays, i.e. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays revealed higher antioxidant activity in in vitro grown plants in comparison to control plants. Essential oil constituent's analysis was also carried out in control and in vitro raised plants. Thirty-one compounds were identified in the oil samples through Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analysis also identified 31 compounds in the essential oil, representing 98.1–98.7% of total oil compositions. The major components of the essential oils were (Z)-ligustilide (51.1–51.5%), (Z)-butylidene phthalide (31.2–31.6%), (E)-butylidene phthalide (2.6–2.9%) and (E)-ligustilide (2.1–1.8%). Genetic stability of in vitro raised plants, evaluated using 20 Inter Simple Sequence Repeats primers, proved true to typeness of in vitro raised plants.

**Keywords** *Angelica glauca* · In-vitro propagation · ISSR · Genetic stability · Antioxidant activity · Essential oil profiling

## Abbreviations

MS	Murashige and Skoog medium
BAP	6-Benzylaminopurine
TDZ	Thidiazuron
IAA	Indole-3-acetic acid
NAA	Naphthaleneacetic acid
Amsl	Above mean sea level
ISSR	Inter simple sequence repeats
GC–MS	Gas chromatography–mass spectrometry
DPPH	1, 1-Diphenyl-2- picrylhydrazyl
FRAP	Ferric reducing antioxidant power

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*Angelica glauca* Edgew. (local name Choru or Gandhrayan; family: Apiaceae) is endemic to India and distributed between 2550 to 3800 m amsl in Uttarakhand, Himachal Pradesh and Jammu and Kashmir (Butola and Badola 2004). It is highly medicinal and aromatic plant species, useful as

stimulant, appetizer, carminative, expectorant, cardioactive, cordial, diaphoretic, and also used in dyspepsia, bilious complaints, stomach difficulty, renal disorders, digestive disorders, lungs disorders, rheumatism, menorrhiza etc. (Anonymous 1985; Agarwal 1986; Kirtikar and Basu 1988; Sharma et al. 1990). Roots of this plant are rich source of high value essential oils, which are useful in modern medicine including aromatherapy (Butola and Vashistha 2013). The species is reported to have huge pharmaceutical and therapeutical importance and anti-inflammatory property due to the occurrence of active constituents like butylidene phthalide and ligustilide (Chung et al. 2012; Feng et al. 2012).

The demands of this species by pharmaceutical industries mostly met through direct collection from natural habitat. Increasing demand of raw material, uncontrolled collection and grazing problems have led to depletion of naturally growing populations (Vashistha et al. 2010), as a result, the species is becoming rare with few habitats and now entered in the list endangered plants of the Himalaya, adopting IUCN criteria (Anonymous 2003).

Over the past several decades in vitro propagation techniques have proved quite beneficial approach for rapid and mass propagation of endangered species, thus helps to conserve the important medicinal plant and reducing the danger of extinction (Nadeem et al. 2001).

The most crucial part of clonal propagation is to maintain genetic authenticity of in vitro-raised plants in relation to their ortet plant (Bairu et al. 2011). The genetic variations can be detected easily through molecular markers e.g. RAPD, ISSR etc, as these markers are sensitive, reliable and time and cost effective method for analysis of clonal stability and are not affected by environmental factors.

Although few studies on *A. glauca* (Bisht et al. 2003; Vashistha et al. 2006, 2010; Butola et al. 2010) are available from IHR focused on species distribution, morphology, phenology and biological activities, however, studies regarding the proposed objectives on the target species have not been carried out yet. There are only two reports on somatic embryogenesis in *A. glauca* (Pandey et al. 2011, Bisht et al. 2015). The published report also lack clonal fidelity and essential oil constituent's analyses, which are very important aspect of plant tissue culture practice.

Keeping the medicinal and conservation importance of *A. glauca*, this study was carried out (1) to develop in vitro propagation protocol for multiple shoot induction (2) to analyze the genetic fidelity of in vitro raised plants using ISSR markers, (3) to evaluate the different phytochemical parameters and antioxidant activities, and (4) to assess the essential oil constituents in control and in-vitro raised plants. The present investigation is the first successful, reproducible and complete report on in-vitro propagation through direct organogenesis of *Angelica glauca*.

Plants of *A. glauca* were collected from Tungnath, Chopta, District Rudraprayag (30°29'21.79"N to 79°13'0.23"E 3444 m amsl) of Uttarakhand, India in the month of September and fetched to the tissue culture laboratory for in vitro propagation studies (Murashige and Skoog 1962). Plant material was verified by the Botanical Survey of India, Dehradun, Uttarakhand, India, and specimen of the plant was deposited in taxonomy laboratory of Forest Research Institute.

Properly sterilized rhizomes of *A. glauca* (20–35 mm long and 10–15 mm diameter) were cultured on MS basal medium (agar 0.8%; w/v and sucrose 3%; w/v; pH 5.8). Cultures were kept at  $22 \pm 2$  °C in 16/8 h light/dark (Philips 40 W; 42.0 and 60.0  $\mu\text{mol}/\text{m}^2/\text{s}$  irradiance inside and outside the culture flasks, respectively) cycle on racks. Sub-culturing was done after 2–3 weeks interval to get best possible growth.

MS medium supplemented with different PGRs (BAP, TDZ and IAA) at various concentrations (Table 1) were tested either individually or in combination for multiple shoot formation. Propagated shoots (approximately 3 to 4 cm long) were transferred on MS medium with combination of IAA (0.0–1.0  $\mu\text{M}$ ) and NAA (0.0–0.5  $\mu\text{M}$ ) for root induction (Table 2). In vitro developed rooted plants were transplanted in tharmacol cups filled with soil, sand and compost (1:1:1) and further kept in green house for one month and survival percentage was calculated. All the experiments were repeated twice and each treatment had 10 replicates. The data were represented as mean with standard error (Mean  $\pm$  SE). The data were analyzed using Statistica for Windows v.6.0 software package (StatSoft). Significant differences were assessed using one-way analysis of variance applying Tukey's test ( $p < 0.05$ ).

Genetic stability analysis was carried out to compare in vitro propagated plants and mother plant of *A. glauca* using ISSR markers. Three different DNA samples (T1, T2 and T3) from three groups (each group contains 25 in vitro raised plants) of in vitro raised plants, were used to check the genetic integrity of plants. DNAs isolated from ten plants (from each group) were mixed and then sample were taken. Juvenile leaves (500 mg) were used for DNA extraction according the method described by Doyle and Doyle (1987).

ISSR analysis was carried out using 20 primers (16–17 mer; set # 9; UBC, Vancouver, Canada) following the method of Rawat et al. (2013a). Amplified products of ISSR analyses were separated through agarose gel electrophoresis and visualized under UV light in Gel Documentation System (GelDoc-It Imaging System, UVP Ltd, Cambridge, UK). Only deeply stained, clear and reproducible bands were scored for analysis. Polymorphic bands (if any) were calculated on the basis of binary character; scored 1 for presence and 0 for absence. Similarity index was calculated through Dice coefficient of similarity (Nei and Li 1979).

**Table 1** Effect of different concentration of plant growth regulators on in vitro regeneration from rhizome explants of *A. glauca* after 4 weeks of culture

Plant Growth Regulators ( $\mu\text{M}$ )			No. of shoots	Shoot length (cm)	No. of leaves
BAP	TDZ	IAA			
0.0	0.0	0.0	1.0 $\pm$ 0.1 <sup>q</sup>	2.6 $\pm$ 0.1 <sup>n</sup>	1.9 $\pm$ 0.1 <sup>p</sup>
1.0	0.0	0.0	2.1 $\pm$ 0.2 <sup>p</sup>	2.3 $\pm$ 0.1 <sup>o</sup>	3.8 $\pm$ 0.3 <sup>h</sup>
2.0	0.0	0.0	2.6 $\pm$ 0.2 <sup>m</sup>	2.6 $\pm$ 0.1 <sup>n</sup>	3.2 $\pm$ 0.2 <sup>j</sup>
4.0	0.0	0.0	3.1 $\pm$ 0.2 <sup>j</sup>	2.8 $\pm$ 0.3 <sup>m</sup>	3.0 $\pm$ 0.1 <sup>l</sup>
8.0	0.0	0.0	3.2 $\pm$ 0.3 <sup>i</sup>	3.5 $\pm$ 0.3 <sup>i</sup>	4.2 $\pm$ 0.3 <sup>e</sup>
10.0	0.0	0.0	3.2 $\pm$ 0.3 <sup>i</sup>	3.6 $\pm$ 0.1 <sup>h</sup>	3.0 $\pm$ 0.1 <sup>l</sup>
1.0	0.0	0.1	2.8 $\pm$ 0.3 <sup>l</sup>	3.4 $\pm$ 0.2 <sup>j</sup>	2.8 $\pm$ 0.1 <sup>m</sup>
2.0	0.0	0.1	2.8 $\pm$ 0.2 <sup>l</sup>	4.0 $\pm$ 0.3 <sup>d</sup>	3.1 $\pm$ 0.2 <sup>k</sup>
4.0	0.0	0.1	3.5 $\pm$ 0.3 <sup>g</sup>	4.0 $\pm$ 0.3 <sup>d</sup>	3.8 $\pm$ 0.3 <sup>h</sup>
8.0	0.0	0.1	5.8 $\pm$ 0.5 <sup>a</sup>	4.8 $\pm$ 0.4 <sup>a</sup>	5.2 $\pm$ 0.4 <sup>d</sup>
10.0	0.0	0.1	4.2 $\pm$ 0.3 <sup>c</sup>	4.1 $\pm$ 0.4 <sup>c</sup>	5.4 $\pm$ 0.5 <sup>c</sup>
1.0	0.0	0.5	4.0 $\pm$ 0.3 <sup>d</sup>	3.8 $\pm$ 0.3 <sup>f</sup>	3.8 $\pm$ 0.4 <sup>h</sup>
2.0	0.0	0.5	4.2 $\pm$ 0.2 <sup>c</sup>	3.9 $\pm$ 0.4 <sup>e</sup>	4.1 $\pm$ 0.4 <sup>f</sup>
4.0	0.0	0.5	3.8 $\pm$ 0.4 <sup>e</sup>	3.8 $\pm$ 0.4 <sup>f</sup>	4.0 $\pm$ 0.5 <sup>g</sup>
8.0	0.0	0.5	4.4 $\pm$ 0.4 <sup>b</sup>	3.9 $\pm$ 0.4 <sup>e</sup>	6.2 $\pm$ 0.7 <sup>a</sup>
10.0	0.0	0.5	3.6 $\pm$ 0.4 <sup>f</sup>	3.9 $\pm$ 0.5 <sup>e</sup>	5.6 $\pm$ 0.5 <sup>b</sup>
0.0	0.5	0.1	2.6 $\pm$ 0.1 <sup>m</sup>	4.0 $\pm$ 0.5 <sup>d</sup>	2.4 $\pm$ 0.1 <sup>o</sup>
0.0	1.0	0.1	2.4 $\pm$ 0.1 <sup>n</sup>	4.2 $\pm$ 0.4 <sup>b</sup>	2.6 $\pm$ 0.1 <sup>n</sup>
0.0	2.0	0.1	3.1 $\pm$ 0.4 <sup>j</sup>	3.7 $\pm$ 0.3 <sup>g</sup>	3.0 $\pm$ 0.3 <sup>l</sup>
0.0	4.0	0.1	3.0 $\pm$ 0.3 <sup>k</sup>	4.0 $\pm$ 0.4 <sup>d</sup>	3.4 $\pm$ 0.3 <sup>i</sup>
0.0	0.5	0.5	3.4 $\pm$ 0.3 <sup>h</sup>	3.2 $\pm$ 0.2 <sup>k</sup>	2.6 $\pm$ 0.1 <sup>n</sup>
0.0	1.0	0.5	2.3 $\pm$ 0.1 <sup>o</sup>	3.2 $\pm$ 0.1 <sup>k</sup>	3.1 $\pm$ 0.2 <sup>k</sup>
0.0	2.0	0.5	2.8 $\pm$ 0.2 <sup>l</sup>	2.8 $\pm$ 0.1 <sup>m</sup>	2.6 $\pm$ 0.1 <sup>n</sup>
0.0	4.0	0.5	3.2 $\pm$ 0.2 <sup>i</sup>	2.9 $\pm$ 0.2 <sup>l</sup>	3.0 $\pm$ 0.1 <sup>l</sup>

Values represent mean $\pm$ SE of ten replicates/treatment and all the experiments were repeated twice. Means followed by the same letter in the column are not significantly different as indicated by Tukey–Kramer HSD ( $p \leq 0.05$ )

For the analysis of total phenol, tannin and flavonoids, the powdered sample of dried roots (1 g), of 1 year old in vitro raised plants and naturally growing (control) plant of same age, was mixed with 5 ml of 80% methanol and ground carefully in Pestle-Mortar. The prepared homogenate was centrifuged for 15 min at 8000 rpm and the supernatant was collected. The residue washed with 50 ml of 80% methanol. The total filtrate (methanolic) was then stored at 4 °C until used. The methanolic extract was used for the analysis of total phenol, flavonoid, tannins and antioxidant activity. Total phenolic and tannin content were analyzed following the protocol of Swain and Hillis (1959). The estimation of total phenolic content, tannin and flavonoids were expressed as mg gallic acid equivalent (GAE) per g of dry weight of sample, mg tannic acid equivalent (TAE) per g dry weight of sample and mg quercetin equivalent per g sample, respectively.

Antioxidant activity was measured through two different in vitro assays [2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay]. The free radical scavenging ability of methanolic extract of *A. glauca* against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was calculated following the procedure given by Braca et al. (2001). Free radical scavenging ability was calculated as follows:

$$\% \text{ Scavenging} = (A_0 - A_1) / A_0 \times 100\%$$

where  $A_0$  is absorbance of the standard and  $A_1$  is absorbance of the sample.

DPPH radical scavenging activity of the species was estimated at different concentration ranging from 250 to 1000  $\mu\text{g}$ , and results expressed in mM ascorbic acid equivalent (AAE) per g dry weight of sample. The FRAP assay was performed using the modified methods of Wong et al. (2006) and Benzie and Strain (1996). The FRAP value was calculated using ascorbic acid as a standard (10–100  $\mu\text{g}$ ; Sigma USA). The results were expressed in mM ascorbic acid equivalent (AAE) per g dry weight of sample.

Essential oil constituent analysis was carried out using the roots of 1 year old tissue culture raised plants growing in the field station at Tungnath and naturally growing plant of the same age (control). Analysis was done following the method of Purohit et al. (2015). GC analysis examined using Agilent (HP7890 GC) gas chromatograph (Flame Ionization detector and HP-5 fused silica column; 30 m $\times$ 0.32 mm, 0.25  $\mu\text{m}$  film thickness). The sample (0.2  $\mu\text{l}$ ) was injected directly into the column. During analysis Nitrogen was used as a carrier gas. The temperature of injector and detector were maintained at 210 and 230 °C, respectively, and column oven temperature was programmed from 60 to 220 °C with an increase in rate of 3 °C/min. In case of GC-MS analyses, Agilent mass spectrometer (Model 5975C) coupled to an Agilent gas chromatograph with a 60 m $\times$ 0.32 mm, 0.25  $\mu\text{m}$  film thickness column (DB5) was used. The sample size was 0.2  $\mu\text{l}$ , which was directly injected in split less mode. Carrier gas (Helium) flow rate was 1 ml/min. Other conditions were the same as described above under GC analysis. The mass spectrum was taken with a mass range of 40–600 Daltons. The constituents were classified on the ground of retention index (RI), deduced with reference to the homologous series of *n*-alkanes, C<sub>8</sub>–C<sub>24</sub> with co-injection of standards (Sigma Aldrich USA) under same analytical conditions and by matching their recorded mass spectra with the MS library (NIST/Pfleger/Wiley) and available literature (Adams 2009).

Results of the study showed that shoot buds induced within 7 days of inoculation on MS medium (Fig. 1a). After that shoot buds did not show further growth due to absence of plant growth regulator in medium. Therefore, various combinations of plant growth regulators BAP (0.0–10  $\mu\text{M}$ ), TDZ (0.0–4.0  $\mu\text{M}$ ) and IAA (0.0–0.5  $\mu\text{M}$ ) were used for

**Table 2** Effect of auxin supplementation on rooting response of *A. glauca*

Growth regulator + 1/2 MS		Shoot forming roots (%)	Number of days to root	Number of roots/shoot	Root length (cm)
IAA ( $\mu\text{M}$ )	NAA ( $\mu\text{M}$ )				
0.0	0.0	$13.6 \pm 0.8^{\text{h}}$	25–30	$1.3 \pm 0.2^{\text{h}}$	$2.6 \pm 0.8^{\text{i}}$
0.1	0.0	$42.3 \pm 1.8^{\text{f}}$	20–32	$2.1 \pm 0.2^{\text{f}}$	$2.9 \pm 1.0^{\text{h}}$
0.5	0.0	$44.6 \pm 2.1^{\text{f}}$	20–28	$2.1 \pm 0.2^{\text{f}}$	$3.8 \pm 1.1^{\text{g}}$
1.0	0.0	$63.2 \pm 2.7^{\text{d}}$	22–30	$2.6 \pm 0.2^{\text{e}}$	$2.6 \pm 0.8^{\text{i}}$
0.0	0.1	$36.6 \pm 1.7^{\text{g}}$	20–28	$1.2 \pm 0.1^{\text{i}}$	$1.6 \pm 0.1^{\text{j}}$
0.1	0.1	$66.3 \pm 2.3^{\text{c}}$	18–28	$3.6 \pm 0.4^{\text{b}}$	$5.6 \pm 2.4^{\text{c}}$
0.5	0.1	$73.4 \pm 2.7^{\text{b}}$	18–30	$3.2 \pm 0.3^{\text{c}}$	$6.2 \pm 2.2^{\text{b}}$
1.0	0.1	$38.6 \pm 1.8^{\text{g}}$	18–28	$2.1 \pm 0.2^{\text{f}}$	$4.1 \pm 1.1^{\text{f}}$
0.0	0.5	$36.4 \pm 1.4^{\text{g}}$	25–30	$2.1 \pm 0.2^{\text{f}}$	$5.2 \pm 1.2^{\text{d}}$
0.1	0.5	$83.7 \pm 3.4^{\text{a}}$	20–28	$4.2 \pm 0.5^{\text{a}}$	$6.2 \pm 2.5^{\text{b}}$
0.5	0.5	$56.6 \pm 2.6^{\text{e}}$	20–26	$1.4 \pm 0.2^{\text{g}}$	$4.4 \pm 1.2^{\text{c}}$
1.0	0.5	$68.2 \pm 2.8^{\text{c}}$	22–28	$2.8 \pm 0.3^{\text{d}}$	$6.4 \pm 1.3^{\text{a}}$

Values represent mean  $\pm$  SE of ten replicates/treatment and all the experiments were repeated twice. Means followed by the same letter in the column are not significantly different as indicated by Tukey–Kramer HSD ( $p \leq 0.05$ )



**Fig. 1** In vitro propagation of *Angelica glauca*. **a** Shoot initiation on MS medium. **b** and **c** Elongation and multiplication of shoot on MS media supplemented with 8.0  $\mu\text{M}$  BAP and 0.1  $\mu\text{M}$  IAA. **d** Rooting

in MS medium supplemented with 0.1  $\mu\text{M}$  IAA and 0.5  $\mu\text{M}$  NAA. **e** Three months old hardened plant; bar = 1 cm

shoot multiplication and elongation (Table 1). The maximum shoot production was obtained in MS medium containing supplemented with 8.0  $\mu\text{M}$  BAP (8.0  $\mu\text{M}$ ) and 0.1  $\mu\text{M}$  IAA (0.1  $\mu\text{M}$ ) showed maximum shoot production (5.8 shoots per explants; Fig. 1b and c). These results validate with earlier reports on several medicinal plants, where multiple shoot production was recorded on MS medium supplemented with BAP at higher concentration in comparison

to auxin (Rawat et al. 2013a; Verma et al. 2014). However, continuous increasing amount of BAP affects adversely the initiation and growth of shoots. Findings of the current study also showed that the lower concentration of IAA with comparatively higher concentration of BAP resulted better shoot multiplication. Also BAP was found more effective for shoot induction in comparison to TDZ. Such type of results reported in several plant systems, including *Oldenlandia*

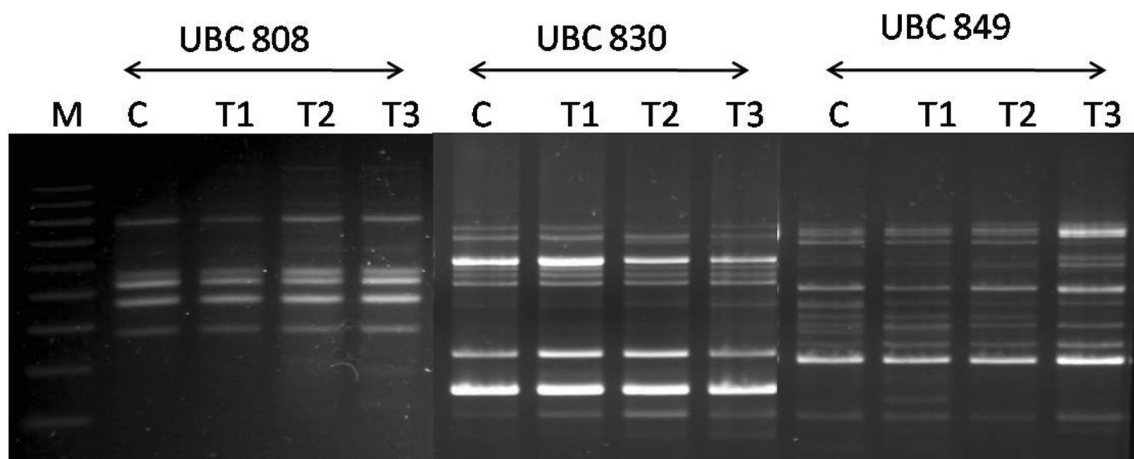
*umbellata* (Siva et al. 2009) *Datura stramonium* (Amiri et al. 2011) and *Plectranthus barbatus* (Thangavel et al. 2011).

For root induction, 2–4 cm long shoots were sub-cultured on MS medium supplemented with IAA or NAA (individual or in combination). The concentration of IAA or NAA affects the root initiation and number of lateral roots (Table 2). Maximum in-vitro root induction frequency (83.7%) was occurred on MS media containing 0.1  $\mu\text{M}$  IAA and 0.5  $\mu\text{M}$  NAA (Fig. 1d). Earlier studies on *Aloe barbadensis* (Samantaray and Maiti 2008), *Oldenlandia umbellata* (Behera et al. 2017) and *A. balforii* (Sharma et al. 2012) also showed that single auxin (IAA or IBA) resulted in less number of roots where as two or more auxin or auxin with coconut milk supplemented in the medium simultaneously induces rooting response at a higher rate (Sharma et al. 2012; Siva et al. 2012). Plantlets (after rooting) were shifted to mixture of soil, sand and compost (1:1:1, v/v), which showed 78.6% survival after 4 week of transfer in the poly-house conditions (Fig. 1e). The plants grew normal well without any phenotypic variation and attained 8–10 cm height within 8 week.

To check the genetic stability analysis of in vitro raised plants of *A. glauca* 20 ISSR primers were analyzed. Out of 20 primers only seven primers produced scorable amplified

products. Total 57 amplified products were recorded from seven ISSR primers; among them 5.26% bands were found to be polymorphic. Minimum 4 (UBC 823) and maximum 11 (UBC 827) number of bands were recorded in a size range of 0.1–2.6 kb. A representative photograph of ISSR analysis is presented in Fig. 2. It was observed that ISSR markers are able to intensify various genomic regions, and better results of genetic similarity or diversity of the tested samples. A similarity matrix calculated from ISSR analyses was 0.987. These results clearly indicate that in vitro developed plants resembled the parent plant at the genetic level. The result of our study showed that in vitro propagation is a reliable biotechnological technique for the production of genetically similar plants.

Total phenolic, tannin and flavonoids content were analyzed in in-vitro propagated plants of *A. glauca*. In vitro raised plants of *A. glauca* showed higher production of total phenol (5.87 mg GAE/ g DW), tannin (1.71 mg TAE/ g DW) and flavonoids (6.32 mg QE/ g DW) in comparison to control plant (phenol 2.36 mg GAE/ g DW); tannin (1.03 mg TAE/ g DW and flavonoids 4.02 mg QE/ g DW; Table 3). Several studies reported beneficial use of different PGRs and elicitors for the enhanced production of



**Fig. 2** ISSR amplification profiles with primers UBC 808, 830 and 849; *M* molecular weight marker; *C* control plant; *T1–T3* tissue culture raised hardened plants

**Table 3** Comparison of total phenol, tannin, flavonoids and antioxidant activity of in vitro raised plants and control plant of *A. glauca*

Plant sample	Total phenol (mg GAE/g DW)	Tannin (mg TAE/g DW)	Flavonoids (mg QE/g DW)	Antioxidant activity (mM AAE/g DW)	
				DPPH	FRAP
In vitro raised	5.87 ± 0.18 <sup>a</sup>	1.71 ± 0.1 <sup>a</sup>	6.32 ± 0.3 <sup>a</sup>	61.83 ± 5.8 <sup>a</sup>	6.83 ± 0.9 <sup>a</sup>
Control	2.36 ± 0.1 <sup>b</sup>	1.03 ± 0.1 <sup>a</sup>	4.02 ± 0.2 <sup>b</sup>	53.0 ± 4.6 <sup>b</sup>	4.98 ± 0.7 <sup>b</sup>

Values represent mean ± SE of 10 replicates/treatment and all the experiments were repeated twice. Means followed by the same letter in the column are not significantly different as indicated by Tukey–Kramer HSD ( $p \leq 0.05$ )

secondary metabolites (Shinde et al. 2010; Giri et al. 2012; Rawat et al. 2013b).

Antioxidant activity of *A. glauca*, measured by two different in vitro assays i.e. DPPH and FRAP, revealed a significant variation ( $p < 0.05$ ) between in vitro raised and control plants (Table 3). In case of DPPH assay in vitro raised plants and control showed 61.83 and 53.0 mM AAE/ g DW, respectively, where as in FRAP assay antioxidant activity was 6.83 and 4.98 mM AAE/ g DW in in vitro raised and control plants, respectively (Table 3). Previous studies reported the antioxidant activity in methanolic extracts of in vitro multiplied plantlets e.g. *Aloe arborescence* (Amoo et al. 2012), *Ceropegia santapau* (Chavan et al. 2014) and *Dendrobium thyrsiflorum* (Bhattacharyya et al. 2015) possess high antioxidant activity. The antioxidant potential may be due to the presence of high amount of polyphenols, flavonoids, tannins and alkaloids.

*Angelica glauca* roots yielded 0.5 and 1.9% (v/w) essential oils in control and in vitro raised plants, respectively. GC and GC–MS analyses revealed thirty-one components in the oils from control (98.1) and in vitro (98.7) raised plants (Table 4). Results based on GC-MS analysis indicated similarity in the essential oil profile of the mother plant and in vitro raised plants. It is confirm that the method followed to produce the in vitro raised plants is able to maintain the identical metabolic profile of the mother plant and, hence, can be utilize for large scale production of true-to-type plants of *A. glauca*. The main constituents present in both oils are phthalides and ligustilides. It consists of 87.4 and 87.8% in control and in vitro raised plants, respectively. The major oil components in the oils of *A. glauca* were (Z)-ligustilide (51.1 and 51.5%), (Z)-butylidene phthalide (31.2 and 31.6%), (E)-butylidene phthalide (2.6 and 2.9%) and (E)-ligustilide (2.1 and 1.8%) in control and in vitro raised plants, respectively. Studies based on chemical profiling of *A. glauca* confirmed its huge pharmaceutical significance as well as essence and aroma value.

In conclusion, the current study demonstrates an easy, reproducible and affordable protocol for the production of genetically identical *A. glauca* plants, an endangered medicinal plant of Himalayan region. This is the first complete report on micropropagation of *A. glauca* with assessment of genetic stability using ISSR-based DNA fingerprinting profiles. Results also showed that in vitro raised plants are superior in production of active compounds and significantly higher antioxidant potential. The most important essential oil analysis of in vitro raised plants also showed similarity in constituents of both the oil samples (control and in vitro raised plants), which indicates that in vitro propagated plants of *A. glauca* can be helpful in reducing pressure on the existing populations. The findings of the present investigation may be utilized for

**Table 4** Chemical Constituents of *Angelica glauca* roots essential oil

Components	Retention index	Composition %	
		Control	In vitro raised
a-Pinene	935	0.3	0.5
Camphene	946	0.7	0.8
Sabinene	970	0.2	tr
Myrcene	986	0.6	0.8
a-Phellandrene	995	1.5	1.5
p-Cymene	1020	0.3	0.3
b-Phellandrene	1023	1.3	1.4
Limonene	1024	0.2	0.2
Cis-ocimene	1031	0.3	tr
Trans-ocimene	1048	–	0.1
c-Terpenene	1049	0.4	0.4
Linalool	1095	tr	0.1
Borneol	1148	0.2	0.2
Terpinen-4-ol	1174	0.6	0.6
Carveol	1191	0.4	0.5
Citronellol	1212	tr	0.2
Carvone	1239	0.3	0.5
b-Elemene	1393	0.5	0.4
b-Caryophyllene	1425	0.4	0.1
Alloromadendrene	1458	0.8	0.7
Germacrene-D	1484	tr	tr
b-Bisabolene	1494	0.4	0.4
d-Cadinene	1525	tr	0.1
Spathulenol	1577	0.3	0.3
Epi-a-cadinol	1635	0.1	tr
b-Eudesmol	1654	tr	0.1
a-Cadinol	1652	1.3	0.7
(Z)-Butylidene phthalide	1670	31.2	31.6
(E)-Butylidene phthalide	1717	2.6	2.9
(Z)-Ligustilide	1736	51.1	51.5
(E)-Ligustilide	1798	2.1	1.8
Total identified (%)		98.1	98.7

the conservation, commercial propagation and enhance the production of bioactive constituents through incorporating changes at genetic level of this high value, medicinal plant of Himalaya which ought to be endangered.

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## Compliance with ethical standards

**Conflict of interest** The authors have declared that no competing interests exist.

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