



# In vitro Propagation of Endemic Species *Mahonia Jaunsarensis* Ahrendt Through Callus Culture

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## Abstract

*Mahonia jaunsarensis* Ahrendt (Family Berberidaceae) an endemic species was successfully propagated in vitro. An efficient propagation protocol has been developed first time. The callus cultures were established from leaf explants on Murashige and Skoog (MS) medium supplemented with 2,4-Dichlorophenoxyacetic acid (2,4-D; 1  $\mu$ M) and resulted 70% callus induction with green compact callus. When callus was transferred to MS medium containing Thidiazuron (TDZ; 0.75  $\mu$ M), maximum average number of shoot (3.06) produced but shoot length (3.37 cm) and average leaf number (2.87) was increased upon transfer to MS medium containing N6-benzylaminopurine (BA; 6.0  $\mu$ M) plus  $\alpha$ -naphthalene acetic acid (NAA; 0.5  $\mu$ M). In MS medium containing indole-3-butyric acid (IBA; 0.01  $\mu$ M), the maximum rooting percentage (56%) and average root number (2.56) per shoot and root length (3.33 cm) were recorded. The rooted plantlets transferred in vermiculite + garden soil + farmyard manure (1:1:1) with maximum (55%) survival percentage under greenhouse condition. The phytochemical analysis of leaves obtained from tissue culture-raised plants revealed significantly higher levels of alkaloids (berberine and palmatine) than those obtained from wild plants. Similar trends were observed for antioxidant and antimutagenic activities. Results of this study offer a baseline for the conservation and sustainable utilization strategies for *M. jaunsarensis*.

**Keywords** Endemic species · Berberine · Palmatine · Antimutagenic activity

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## Introduction

The genus *Mahonia* (family: Berberidaceae) widely distributed in East and Southeast Asia, Western North America, Central America, and Western South America. In context to the Himalayan region the genus is reported from Nepal, India, Bhutan, China, and Vietnam at an altitude between 1,000–2,400 m. s. l. [1]. *Mahonia* has about 70 species worldwide [2], out of which 13 have been reported from Indian Himalaya [3] and four species (viz. *M. acantifolia*, *M. borealis*, *M. nepalensis*, *M. jaunsarensis*) from Uttarakhand [4], one of these *M. jaunsarensis* is endemic to Uttarakhand [4, 5]. Among the berberidaceae plants, *Mahonia jaunsarensis* Ahrendt is the most valuable woody perennial shrub. The species is endemic, found particularly in the Chakrata region of Dehradun (West Himalayan) and is called “khoru” locally. Only a very small number of populations of this plant grow naturally in oak banj forest at an altitude of 2,300 to 4,300 m. s. l. Local people use this plant for treating fevers, cough, asthma by using its edible fruit, root and stem bark. The species reported to possess antioxidant activity [6] and have diverse medicinal uses, thus requires adequate populations in nature. The natural habitat of *M. jaunsarensis* is diminishing due to increasing anthropogenic activities, long period of dormancy, fungal and insect attack, indiscriminate collection, deteriorating natural ecosystem, etc. Although, Bisht et al. [7] standardized a protocol for vegetative propagation of the species using stem cuttings but unable to produce enough number of plants and required longer response time. Over the years, plant tissue culture has emerged as one of the most effective alternate method for large-scale production, maintaining genetic uniformity and secondary metabolite production [8, 9]. As a result of this, many rare and endangered species have been propagated in the last few decades [10]. Successful in vitro studies have been performed on other species of genus *Mahonia*, namely *M. leschenaultia* [11] and *M. soft carees* [12], however, in vitro propagation protocol for *M. jaunsarensis* along with alkaloid studies of in vitro raised plants have not been conducted yet. Therefore, the present study aimed to (i) develop an effective in vitro regeneration protocol, (ii) analyse the secondary metabolites in in vitro raised and wild plant leaves, and in vitro developed callus, and (iii) evaluate the antioxidant and antimutagenic activity of both wild-grown and in-vitro regenerated plants.

## Materials and Methods

### Sample Collection

Ripened fruits of *M. jaunsarensis* were collected from the natural habitat of Chakrata, Dehradun, Uttarakhand (N 30°45'5.76"; E77°52'10.56"; altitude: 2200 m. s. l.) and the voucher specimens were deposited at the Botanical Survey of India, Dehradun, Uttarakhand (Acc. No.56). All the experiment conducted at G. B Pant National Institute of Himalayan Environment (NIHE), Kosi-Katarmal, Almora, Uttarakhand (India).

### Explant Preparation

Briefly, the seeds were rinsed with running tap water (2–3 times) then washed by distilled water (2–3times). After that, 3–5 drops of surfactant (Tween 20; Hi-Media,

Mumbai, India) were added and washed thoroughly. Treatment with fungicide (Bavistin) solution at (0.1% 0.5%, 0.01%) was applied for 10, 20, 30 min, followed by shaking in a magnetic stirrer. Thereafter, seeds were treated with different concentrations (0.1%, 0.5%, 0.01%) of mercuric chloride ( $\text{HgCl}_2$ ) for 5 min, 10 min, and 15 min under laminar airflow. Finally, seeds were washed with sterile double distilled water for 4–6 times.

### Effect of media

To determine the best culture medium, seeds were inoculated (four seeds per flask) on Murashige and Skoog media (MS) (Murashige and Skoog, 1962) as well as on Woody Plant media (WP) (Lloyd & McCown, 1981). The pH of the medium was adjusted to 5.8 using 1 N NaOH and HCl and the medium was supplemented with 3% (w/v) sucrose and solidified with agar (0.8% w/v) (Hi-media, Mumbai, India). The prepared media was dispensed into the culture tubes and autoclaved at 15 psi for 20 min at 121 °C. Inoculated culture tubes were kept in culture room conditions ( $25 \pm 20$  °C) for the 16-h photoperiod under cool-white fluorescent light.

### Effect of PGRs

The best growth hormone was determined by inoculating seeds in different medium supplemented with gibberellic acid ( $\text{GA}_3$ ; 0.5–10  $\mu\text{M}$ ) and N6-benzylaminopurine (BA; 0.5–10  $\mu\text{M}$ ) (Table 1). After 30 days, leaves of newly germinated seedlings were used as explant for callus induction.

### Callus induction

Callus initiation was achieved by removing young leaves from in vitro produced seedlings and cut into pieces ( $1 \times 1 \text{ cm}^2$ ) before being cultured in MS basal medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar containing N6-benzylaminopurine (BA; 0.5  $\mu\text{M}$ ),  $\alpha$ -naphthalene acetic acid (NAA; 0.1–4  $\mu\text{M}$ ) with various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D; 0.1–4  $\mu\text{M}$ ) either alone or in combination of BA (0.5  $\mu\text{M}$ ). After 30 days of culture, data on callus induction was recorded.

**Table 1** Effect of different concentrations of BA and  $\text{GA}_3$  on in vitro seed germination of *M. jaunsarensis*

PGR concentration ( $\mu\text{M}$ )		Germination	MGT
BA	$\text{GA}_3$	(%)	(Days)
0.5	0	$56.66 \pm 3.33\text{b}$	$29.51 \pm 0.66\text{a}$
1	0	$83.33 \pm 3.33\text{a}$	$23.45 \pm 0.18\text{bc}$
5	0	$46.66 \pm 5.77\text{bc}$	$24.49 \pm 0.19\text{b}$
10	0	$40.00 \pm 5.77\text{c}$	$22.54 \pm 0.28\text{c}$
0	0.5	$50.00 \pm 5.77\text{b}$	$25.67 \pm 2.22\text{a}$
0	1	$76.66 \pm 0.00\text{a}$	$23.22 \pm 0.56\text{c}$
0	5	$40.00 \pm 5.77\text{bc}$	$26.64 \pm 0.29\text{bc}$
0	10	$36.66 \pm 3.33\text{c}$	$23.75 \pm 0.37\text{c}$
0	0	$46.66 \pm 3.33\text{bc}$	$28.44 \pm 0.27 \text{b}$

## Shoot regeneration and proliferation

For the shoot regeneration callus were transferred on MS medium supplemented with different concentrations and concentrations of plant growth regulators including thidiazuron (TDZ; 0.25–1  $\mu\text{M}$ ), N6-benzylaminopurine (BA; 0, 2.0, 4.0, 6.0 and 8.0  $\mu\text{M}$ ),  $\alpha$ -naphthalene acetic acid (NAA; 0.5  $\mu\text{M}$ ) and indole-3-acetic acid (IAA; 0.5  $\mu\text{M}$ ). Data on the percentage of responsive calli, shoot number and shoot length was recorded after eight weeks of culture.

## Root induction

Well-developed microshoots (>5 cm long) were transferred to half MS media supplemented with different concentrations of indole-3-butyric acid (IBA; 0.01–0.1), indole-3-acetic acid (IAA; 0.01–0.1), and  $\alpha$ -naphthalene acetic acid (NAA; 0.01–0.1  $\mu\text{M}$ ). The plantlets were removed from the culture flask after six weeks of culture for data recording.

## Acclimatization

Acclimatization of the well-rooted healthy plantlets was performed after 14 weeks. Roots were removed carefully from culture bottles and washed thoroughly with water to remove the traces of gelling agent (agar). Then, they were transferred into plastic pots (15 cm height and 12 cm diameter). Different substrate types viz. vermiculite, perlite, sand, farmyard manure and garden soil and their combinations were used to identify the optimal conditions for acclimatization of in-vitro raised plantlets. The five substrate combinations namely sand+farmyard manure (1:1), garden soil+farmyard manure (1:1), vermiculite + garden soil+farmyard manure (1:1:1), vermiculite + sand + farmyard manure (1:1:1) were used. All these substrate combinations were sterilized before transplanting the plants, which were then covered with perforated plastic bags (Fig. 3 (i, j, k) and Fig. 3 (i and j) to maintain humidity. Initially, these plantlets were acclimatized in culture room conditions ( $25 \pm 5$  °C, 16/8 light/dark photoperiod) and after four weeks perforated polybags were removed and plants were placed inside glass houses for further growth and development.

## Phytochemical and antioxidant activity

The accumulation of phytochemicals and antioxidant activities in different in vitro raised and wild plant leaves, and in vitro raised callus, were determined following standard methodologies [13].

## Alkaloid quantification

The amount of berberine and palmatine were measured with High Performance Liquid Chromatography (Alliance Waters e2695, Waters, Milford, USA) equipped with photo diode array (PDA 2998) detector as described by Belwal et al. [14]. In the separation, mobile phase consisted of acetonitrile (A) and 0.13% potassium dihydrogen phosphate

with a pH of 2.5 (B) in a 50:50 ratio at 1.0 ml/min for a total run time of 20 min on a SPHERISORB C18 reverse phase column. In both cases, the detection wavelength was set to 345 nm for both alkaloids. Using berberine and palmatine concentrations ranging from 10 to 50 mg/l, standard curves were created for developing linear regression equations, and their authenticity was assessed using regression coefficients ( $R^2$ ). Chemicals were identified by their retention time (Rt) and quantified by their area under the curve (AUC). The results were expressed in milligram per gram of dry weight (mg/g DW).

### Anti-mutagenic activity

Plasmid (pBR322) DNA was tested for DNA damage activity based on the method of Singh et al. [15]. The plasmid DNA (2  $\mu$ L, 180 ng) was damaged under UV radiation for 15 min in the presence of  $H_2O_2$  (3.5%). Indicators like conversion of the plasmid supercoiled (S) DNA to the open-circular (OC) or linear (L) is marked as a sign of DNA damage. The extent of DNA damage and preventive effect of the test samples were analysed on agarose gel (1.0%) in 1X TAE buffer run over 2 h at 50 Volt at room temperature. The band density was calculated under the gel documentation system (UVI pro Platinum ver. 12.9, UK). The following equation was used to calculate the supercoiled DNA intensity:

$$(S\%) = [S \text{ band density}] \setminus (\text{density of } (S + L + OC))] \times 100.$$

The recovery of supercoiled DNA was also calculated with reference to positive control as relative supercoiled DNA (%) = s (%) of test sample/s (%) of positive control.

### Data Analysis

During seed germination, seedlings were monitored regularly, and if the radicle was visually identified, seeds were considered as germinated. The following formulas were used to calculate percentage of germination (GP) and mean germination time (MGT) [16]

$$\text{Germination percentage (GP)} = \text{GN/SN} \times 100$$

$$\text{Mean germination time (MGT)} = (N_1T_1 + N_2T_2 \dots \dots N_nT_n) \text{GN}$$

where, GN – total number of seeds germinated in 40 days, SN – total number of seeds tested, Sn – number of seed germinated each day of observation, N- days for germination,  $N_1T_1$ - number of seed germinated at time  $T_1$ , SL – mean seedling length (mm). The observed data on various parameters was analysed using IBM SPSS Statistics V21.0 software.

## Results and Discussion

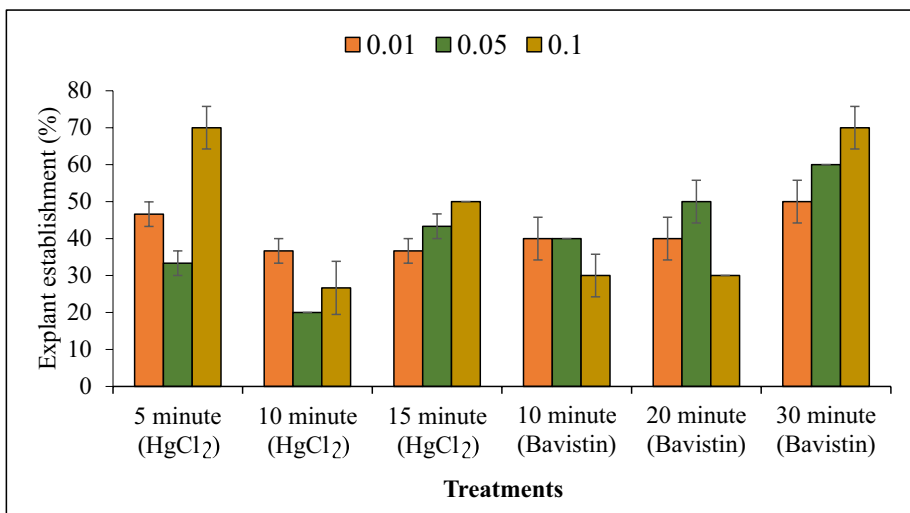
### Sterilization and Explant Establishment

Disinfected seeds were treated with (0.1%, 0.5%, 0.01% of  $HgCl_2$  and Bavistin) for different time durations (5 min, 10 min, and 15 min for  $HgCl_2$ ) and (10 min, 20 min, and 30 min)

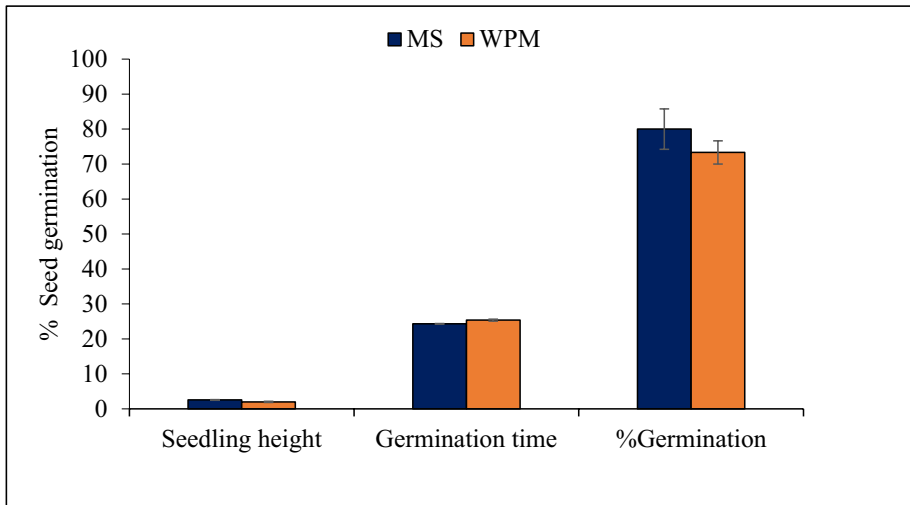
for Bavistin. Among the various pre-treatments used, 0.1%  $\text{HgCl}_2$  for 5 min produced the highest (70%) aseptic cultures (Fig. 1). In addition, 0.1% Bavistin on applying for 30 min resulted maximum aseptic cultures (70.00%). Similar findings were obtained in *Berberis asiatica*, where 0.1%  $\text{HgCl}_2$  for 10 min and Bavistin for 30 min gave best response [17]. Likewise, Brijwal et al. [18] reported application of 0.7% Bavistin and 0.1%  $\text{HgCl}_2$  for 20 min as best treatment for seed sterilization in *Berberis aristata*, Pandey et al. [19] reported 0.5% Bavistin for 30 min and 0.1%  $\text{HgCl}_2$  for 12 min as best seed sterilization treatment for *Berberis chitria*, and Dhar et al. [20] sterilized seeds of *Berberis lycium* with 0.5% Bavistin for 10 min and 0.1%  $\text{HgCl}_2$  for 2 min. Apart from seeds the nodes of *Mahonia leschenaultia* were surface sterilized with 0.1%  $\text{HgCl}_2$  for 5–10 min [11]. Besides, other woody plant species, such as *Cnidioscolus aconitifolius* [21] and *Atriplex taltalensis* [22] were also reported best sterilized with 0.1%  $\text{HgCl}_2$  for 5 min and 10 min respectively.

### Effect of Media on In Vitro Seed Germination in *M. Jaunsarensis*

Germination was observed in all tested media, but their germination rates were different (Fig. 2). The present study found that MS medium yielded the best seed germination response among the used media. In MS medium, with a minimum average time ( $24.31 \pm 0.09$  ds) the maximum percentage of germination ( $80.00 \pm 5.77$  %) and average plant height ( $2.55 \pm 0.08$  cm). While the seedling height in WP medium was comparatively lower than MS medium, the seedling germination percent was ( $73.33 \pm 3.33$  %) with an average germination time of ( $25.37 \pm 0.27$  ds) and an average seedling height of ( $1.99 \pm 0.15$  cm). Similarly, Pandey et al. [19] reported maximum (78.89%) seed germination in WP as compared to MS (50%) in *Berberis chitria*. However, Sharma et al. [23] observed best seed germination (65%) in MS medium in *B. chitria*. The maximum shoot multiplication was also observed in WP medium for *Berberis aristata* [18]. Moreover,



**Fig. 1** Effect of  $\text{HgCl}_2$  and Bavistin concentrations and treatment duration on explant (seed) establishment of *M. jaunsarensis*



**Fig. 2** Effect of media on in vitro seed germination in *M. jaunsarensis*

the best in vitro propagation results for *B. asiatica* were obtained in MS medium by Bisht et al. [17] and Dobhal et al. [24]. MS medium was also found to be the most effective in germination of seeds in *Berberis lycium* [20]. According to the studies, MS is the preferred medium for number of species, including woody ones. However, tissue culture of such woody species as *M. leschenaultia*, and *Mahonia* ‘Soft Caress’ has also been accomplished with other nutrient formulations. In *M. leschenaultia*, Radha et al. [11] used MS, WP and Schenk and Hildebrandt (SH) medium, and found the best shoot multiplication in SH medium. Similarly for *Mahonia* ‘Soft Caress’ Rounsaville et al. [12] tested Gamborg B5 (B5), Quoirin and Lepoivre (QL), and MS medium and reported best shoot multiplication in B5 medium. Other woody plant species such as *Shorea robusta* [25], *Quercus serrata* [26], *Zanthoxylum armatum* [27] and *Salix acmophylla* [28] also propagated in vitro using WP medium.

### Effect of Plant Growth Regulators on Seed Germination

In vitro seed germination response of *M. jaunsarensis* under different treatment was studied. The radical emergence was observed in 12–15 days of inoculation and complete growth of cotyledons was seen between 30 to 35 days. After ten weeks of inoculation seeds of *M. jaunsarensis* showed significantly ( $p < 0.05$ ) higher germination percentage ( $83.33 \pm 3.33$  %) in MS medium supplemented with BA ( $1 \mu\text{M}$ ) with the least germination time ( $23.45 \pm 0.18$  ds) (Table 1). However, in control condition, the lowest germination percentage ( $46.66 \pm 3.33$  %) with an average time of ( $28.44 \pm 0.27$  ds) was observed. Similarly, Sharma et al. [23] reported a maximum (65.46%) seed germination in *B. chitria* using BA (6 mg/l), Bisht et al. [17] also observed higher (50%) germination in *B. asiatica* when treated with BA ( $1 \mu\text{M}$ ). Similarly, Belwal et al. [16] studied seed germination response of *B. aristata* and *B. jaeshkeana* under six treatments (viz.  $\text{GA}_3$ , salicylic acid, thiourea, vermiwash, and cow urine) and found that vermiwash and  $\text{GA}_3$  produced the best seed germination responses. Gibberellic acid treatment promotes the synthesis and production of hydrolases, especially amylase, which breaks down starch into maltose (a chain of two

glucose molecules), thereafter, maltose breaks down into glucose, which is used for the growth of plumule and radicle hence, result in the germination of seeds [28, 29]. In addition, it has been well documented that BA independently serves as a better cytokinin for shoot induction [30–32].

### Callus Induction from Leaf

In order to induce callus from leaf explants, different concentrations of cytokinin and auxin were used individually or in combination (Table 2). Explants were unable to form callus when cultured on MS medium without auxin or cytokinin (control). After seven-week incubation period the formation of compact callus was observed in plant growth regulator containing media. It was observed that the highest percentage of compact green callus formation ( $76.66 \pm 3.33\%$ ) occurred in MS medium supplemented with 2,4-D+BA ( $1.0+0.5 \mu\text{M}$ ), followed by ( $70.00 \pm 0.00\%$ ) in MS medium supplemented with 2, 4-D ( $0.1 \mu\text{M}$ ) (Fig. 3, b, c, d). Callusing percentage was observed low ( $20.00 \pm 0.00\%$ ) for the medium containing NAA + BA ( $3.0+0.5 \mu\text{M}$ ) and NAA + BA ( $4.0+0.5 \mu\text{M}$ ) and produced black compact textured callus (Fig. 3, f). In all treatments, a decline in callus induction was observed with an increase in concentration of PGRs (viz. 2,4-D, BA and NAA) from  $2 \mu\text{M}$  to  $4 \mu\text{M}$ . The combination of 2,4-D and cytokinins known to improve callus regeneration in both monocots and dicots Mehta et al. [33] Bisht et al. [17]. The results of our study are in line with Bisht et al. [17], Rawat et al. [34] and Santos et al. [35] who observed that

**Table 2** Effect of different PGRs concentrations on callus induction from in vitro germinated *M. jaunsarensis* seedling leaves

PGR concentration ( $\mu\text{M}$ )	PGR concentration ( $\mu\text{M}$ )			Callusing (%)	Morphogenetic appearance
	2,4-D	NAA	BA		
0.1				$50.00 \pm 0.00\text{bcd}$	Green, Friable
0.5				$56.66 \pm 3.33\text{ab}$	Green, compact
1				$70.00 \pm 0.00\text{a}$	Green, compact
2				$46.66 \pm 3.33 \text{cd}$	Light Green, friable
3				$46.66 \pm 3.33 \text{cd}$	Yellow, Green
4				$43.33 \pm 3.33\text{de}$	Yellow, friable
	0.1	0.5		$36.66 \pm 3.33\text{ef}$	Brown, compact
	0.5	0.5		$30.00 \pm 0.00 \text{fg}$	White friable
	1	0.5		$30.00 \pm 0.00 \text{fg}$	Brown, compact
	2	0.5		$23.33 \pm 3.33\text{gh}$	Brown, compact
	3	0.5		$20.00 \pm 0.00 \text{h}$	Black, compact
	4	0.5		$20.00 \pm 0.00 \text{h}$	Black, compact
0.1		0.5		$56.66 \pm 3.33\text{ab}$	Green, friable
0.5		0.5		$60.00 \pm 0.00\text{a}$	Green, friable
1		0.5		$76.66 \pm 3.33\text{a}$	Green, friable
2		0.5		$53.33 \pm 3.33\text{abc}$	Yellow compact
3		0.5		$53.33 \pm 3.33\text{abc}$	Light yellow, compact
4		0.5		$46.66 \pm 3.33 \text{cd}$	Brown, compact

Values are mean  $\pm$  standard error; Mean values followed by the same letter(s) in a column are not significantly different ( $p < 0.05$ ) based on DMRT



cytokinin and auxin synergistically induce better callus growth in *Berberis asiatica*, *Aconitum violaceum*, and *Piper permucronatum*.

### Plant Regeneration Through Callus

The healthy callus taken after 6 weeks of culture and transferred to the shoot regeneration medium. Different PGRs exhibited different responses and data related to leaf number, shoot length, and shoot number were observed for shoot regeneration (Table 3). Among the various treatment, MS medium containing TDZ (0.75  $\mu\text{M}$ ) resulted significantly ( $p < 0.05$ ) higher shoot number ( $3.06 \pm 0.03$ ), however, best average shoot length ( $3.37 \pm 0.13$  cm) and average leaf number ( $2.87 \pm 0.23$ ) were recorded in combination of BA+NAA (6+0.5  $\mu\text{M}$ ) (Fig. 3, d) Whereas, lowest average number of shoots ( $0.70 \pm 0.00$ ), average shoot length ( $0.11 \pm 0.01$  cm) and average leaf number ( $0.22 \pm 0.06$ ) were observed in BA+IAA (2.0+0.5  $\mu\text{M}$ ). The species grown in vitro responded well to TDZ (0.75  $\mu\text{M}$ ) and was considered the most effective medium for promoting shoot initiation. Application of TDZ has been reported effective in growing endangered dicotyledonous plants from the Himalayas, including recalcitrant and woody plant species [36–38]. However, for shoot length and shoot multiplication, TDZ was not found to be the best cytokinin since it inhibited growth. Similarly, in *Mahonia* 'Soft Caress', the shoot length significantly decreased after applying TDZ. Many medicinal plants have demonstrated a synergistic effect of hormones on shoot length and shoot multiplication in *Dendrocalamus latiflorus* [36], *Zanthoxylum armatum* [37], *Bambusa tulda* Roxb and *Dendrocalamus stocksii* Munro [39].



**Fig. 3** In vitro propagation of *Mahonia jaunsarensis*. (a,b,c) in vitro seed germination in 25 days; (d) callus induction after seven weeks; (e, f) shoot induction after 5 weeks; (g) root induction after 6 weeks; (h) well rooted plantlet ready for hardening; (i) in vitro raised plant after transfer to cups (kept inside culture room covered with transparent polybags to maintain humidity); (j and k) plants transferred to potting mixture; (l) well-developed in vitro grown plant in glasshouse condition after 120 days of transfer to nursery bags

**Table 3** Effect of different concentrations and combination of PGRs on indirect organogenesis in *M. jaunsarensis*

Treatments	PGR concentration ( $\mu\text{M}$ )				Shoot numbers	Shoot length (cm)	Leaf numbers
	TDZ	BAP	IAA	NAA			
T1	0.25				$2.06 \pm 0.03\text{d}$	$0.58 \pm 0.14\text{d}$	$1.31 \pm 0.06\text{cde}$
T2	0.5				$2.30 \pm 0.05\text{cd}$	$1.65 \pm 0.29\text{c}$	$1.03 \pm 0.02\text{e}$
T3	0.75				$3.06 \pm 0.03\text{a}$	$0.60 \pm 0.25\text{d}$	$1.34 \pm 0.16\text{cde}$
T4	1				$2.13 \pm 0.03\text{cd}$	$0.31 \pm 0.06\text{d}$	$1.53 \pm 0.12\text{cd}$
T5		2	0.5		$0.70 \pm 0.00\text{e}$	$0.11 \pm 0.01\text{d}$	$0.22 \pm 0.06\text{f}$
T6		4	0.5		$0.76 \pm 0.03\text{e}$	$0.16 \pm 0.02\text{d}$	$1.14 \pm 0.04\text{de}$
T7		6	0.5		$0.86 \pm 0.03\text{e}$	$0.45 \pm 0.23\text{d}$	$1.14 \pm 0.04\text{de}$
T8		8	0.5		$0.80 \pm 0.05\text{e}$	$0.14 \pm 0.02\text{d}$	$1.62 \pm 0.08\text{c}$
T9		2		0.5	$2.13 \pm 0.08\text{cd}$	$1.52 \pm 0.19\text{c}$	$2.51 \pm 0.10\text{ab}$
T10		4		0.5	$2.22 \pm 0.01\text{cd}$	$1.60 \pm 0.16\text{c}$	$2.5 \pm 0.30\text{ab}$
T11		6		0.5	$2.67 \pm 0.03\text{b}$	$3.37 \pm 0.13\text{a}$	$2.87 \pm 0.23\text{a}$
T12		8		0.5	$2.36 \pm 0.21\text{c}$	$2.45 \pm 0.19\text{b}$	$2.28 \pm 0.00\text{b}$
Control	0	0	0	0	$0.00 \pm 0.00$	$0.00 \pm 0.00$	$0.00 \pm 0.00$

## In Vitro Rooting

The rooting response of micro shoots under different auxin (IAA, IBA and NAA) treatments responded differently. The significantly ( $p < 0.05$ ) higher rooting percentage ( $56.66 \pm 3.33\%$ ), average root number ( $2.53 \pm 0.13$ ), average root length ( $3.33 \pm 0.11\text{ cm}$ ) were recorded in IBA ( $0.01\ \mu\text{M}$ ) treatments, while, minimum rooting ( $23.33 \pm 3.33\%$ ), average root number ( $0.29 \pm 0.10$ ), average root length ( $0.13 \pm 0.02\text{ cm}$ ) were observed in NAA ( $0.01\ \mu\text{M}$ ) treatments (Table 4). The in vitro rooting of *M. jaunsarensis* shoots are shown in Fig. 3, g & h. In *Mahonia* 'Soft Caress' microcuttings treated with IBA ( $8\ \mu\text{M}$ ) and kept in dark showed the highest rooting percentage (37%) [12]. In *Mahonia leschenaultia* Radha et al. [11] also found the best rooting (78%) response in IBA ( $1\text{ mg/l}$ ). Similar results were observed for *Berberis chitriya* with (100%) rooting in IBA ( $100\ \mu\text{M}$ ) [19] and (71%) rooting for *B. aristata* in IBA ( $50\ \mu\text{M}$ ) [18]. However, Bisht et al. [17] reported higher rooting percentage (70%) in IAA ( $0.05\ \mu\text{M}$ ) for *B. asiatica*. Likewise, Sharma et al. [23] found that the combination of NAA ( $1.0\text{ mg/l}$ ), IAA ( $0.5\text{ mg/l}$ ), and IBA ( $0.5\text{ mg/l}$ ) produced the maximum rooting (76%) in *B. chitriya*. Several studies elsewhere also reported IBA as the most suitable auxin for rooting in a wide variety of plant species [40, 41].

## Acclimatization

Well-developed in vitro rooted plants of *M. jaunsarensis* were transferred to different potting mixtures for further growth and development (Fig. 3, i, j & k). The highest survival percentage (55%) was observed in potting mixture containing vermiculite, garden soil and farmyard (1:1:1), whereas minimum (30%) survival percentage was observed in potting mixture containing sand and farmyard manure (1:1) (Table 5).

**Table 4** Effect of different auxin concentrations on *M. jaunsarensis* root induction of in vitro raised shoots

Treatments in ( $\mu\text{M}$ )			Rooting (%)	Average root number	Average root length (cm)
IBA	IAA	NAA			
0.01			$56.66 \pm 3.33\text{a}$	$2.53 \pm 0.13\text{bc}$	$3.33 \pm 0.11\text{a}$
0.05			$46.66\text{a} \pm 3.33\text{b}$	$2.39 \pm 0.08\text{a}$	$3.27 \pm 0.06\text{a}$
0.1			$46.66 \pm 3.33\text{ab}$	$2.78 \pm 0.34\text{a}$	$3.48 \pm 0.15\text{a}$
	0.01		$30.00 \pm 5.77\text{cd}$	$1.51 \pm 0.13\text{b}$	$2.64 \pm 0.02\text{b}$
	0.05		$26.66 \pm 3.33\text{cd}$	$1.32 \pm 0.00\text{bc}$	$2.55 \pm 0.18\text{b}$
	0.1		$26.66 \pm 3.33\text{cd}$	$0.9 \pm 0.40\text{cd}$	$2.51 \pm 0.14\text{b}$
		0.01	$23.33 \pm 3.33\text{d}$	$0.29 \pm 0.10\text{d}$	$0.13 \pm 0.021\text{d}$
		0.05	$40.00 \pm 10.00\text{bc}$	$0.61 \pm 0.11\text{d}$	$1.03 \pm 0.09\text{c}$
		0.1	$36.66 \pm 3.33\text{bcd}$	$0.36 \pm 0.04\text{d}$	$0.16 \pm 0.01\text{d}$
Control			$00.00 \pm 00.00\text{e}$	$0.00 \pm 0.00\text{e}$	$0.00 \pm 0.00\text{e}$

### In Vitro Phytochemical production

In vitro raised leaves of *M. jaunsarensis* (15-week-old) had significantly higher total phenol ( $36.65 \pm 0.45$  mg GAE/g FW), tannin ( $10.35 \pm 0.15$  mg TAE/g FW) content, and ABTS ( $19.45 \pm 0.75$  mM AAE/100 g FW), FRAP activity ( $3.63 \pm 0.11$  mM AAE/100 g FW) activities. However, DPPH ( $8.90 \pm 0.20$  mM AAE/100 g FW) activity was significantly lower in in vitro raised plant leaves as compared to wild plant leaves (Table 6). Although, flavonoid content was observed similar in both in vitro ( $2.22 \pm 0.05$  mg QE/g FW) and wild ( $2.01 \pm 0.05$  mg QE/g FW) leaf samples. The HPLC results indicate that the in vitro raised plant leaves have more alkaloid content [viz. berberine ( $3.54 \pm 0.00$  mg/g FW) and palmatine ( $0.58 \pm 0.00$  mg/g FW)] than wild plant leaves. In the present study, in vitro-regenerated plant samples showed higher phytochemical, phenolic, tannin, and flavonoid content, which can be correlated to the exogenous supply of different PGRs in in vitro condition. The PGRs are probable precursors for the conversion of phytochemicals into active forms during stress or adverse environmental conditions, where they enhance phytochemicals [42]. Besides, the phytochemical contents may vary depending on factors such as the climate, soil texture, habitat, aspect, and slope, etc. [17]. A similar finding were observed

**Table 5** Effect of different fertilizers on the survival of *M. jaunsarensis*

SN	Different mixture	Ratio of potting mixture	Survival percentage of hardened plants
1	Sand + Farmyard manure	1:1	30%
2	Garden Soil + Farmyard manure	1:1	40%
3	Vermiculite + Garden Soil + Farmyard manure	1:1:1	55%
4	Vermiculite + Sand + Farmyard manure	1:1:1	50%

**Table 6** Phytochemical, antioxidant activity of in vitro leaves and wild leaves extracts of *M. jaunsarensis*

Phytochemical analysis	In vitro leaves	Wild leaves	Callus
Total phenolic content (mg GAE/g FW)	36.65 ± 0.45	21.65 ± 0.05 -	
Tannin content (mg TAE/g FW)	10.35 ± 0.15	09.35 ± 0.45	-
Flavonoid content (mg QE/g FW)	02.22 ± 0.05	02.01 ± 0.05 -	
Antioxidant activity			
ABTS (mM AAE/g FW)	19.45 ± 0.75	16.2 ± 1.00 -	
DPPH (mM AAE/g FW)	08.90 ± 0.20	18.21 ± 0.78 -	
FRAP (mM AAE/g FW)	03.63 ± 0.11	02.55 ± 0.04 -	
Alkaloid			
Berberine (mg/g FW)	03.54 ± 0.00	0.58 ± 0.00	3.55 ± 0.00
Palmatine (mg/g FW)	02.21 ± 0.11	0.13 ± 0.00	0.13 ± 0.00
Supercoiled DNA protection activity (%)	39.66 ± 1.47	61.80 ± 2.28	-

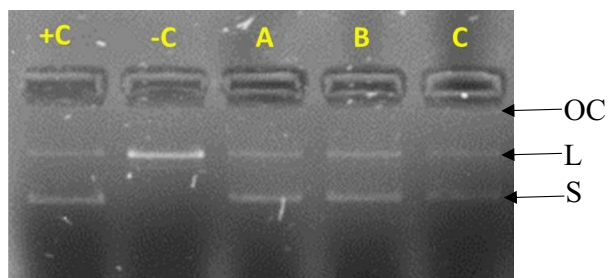
Values are mean ± standard error

in *Habenaria edgeworthii* [43], *Justicia gendarussa* [44], *Atropa acuminata* [45], *Berberis asiatica* [17], where in vitro raised plants had higher phenolic content than wild plants.

### Anti-Mutagenic Activity

In vitro raised plant leaf extract of *Mahonia jaunsarensis* showed considerable anti-mutagenic activity. The damaged pBR322 plasmid DNA when treated with extracts of in vitro raised plant leaves at concentration of 10 µg/ µL, showed 39.66% recovery of damaged plasmid DNA (Fig. 4). Further, the efficaceae of the plant extract was compared with the standard marker antioxidant compounds (i.e., gallic acid) at a concentration of 4 µg/µL and mother plant extract at a concentration of 10 µg/ µL, which showed recovery percent of 64.84% and 61.80% respectively. Overall, the standard marker compounds, gallic acid showed maximum anti-mutagenic activity followed by mother plant leaf extract (Fig. 4), while tissue culture gown plant leaf extract showed comparatively low activity. Several Himalayan medicinal plants have also been studied for DNA damage protection activity, including *Ashtvarga* species (*Habenariya edgeworthi*, *H.intermedia*, *Malaxcis acuminata*, *M. mucifera*, *Polyganatum cirrifolium*, *P. verticillatum*, *Roscea procera* and *Lillum*

**Fig. 4** Anti-mutagenic activity of different extracts of *M. jaunsarensis*. (+C) Positive control; (-C) Negative control; (A) Gallic acid (4µg/ µl; 64.84% Recovery percent), (B) Mother plant leaf extract (10µg/ µl; 61.80% Recovery percent), (C) In vitro growing plant leaf extract (10µg/ µl; 39.66% Recovery percent)



*polyphyllum* [46], *Quercus serrata* [26], *Origanum vulgare* [13] and *B. asiatica* [17] The antimutagenic properties of the species can be correlated to its polyphenolic content and antioxidant properties.

## Conclusion

This study is the first report on in vitro propagation of *M. jaunsarensis* which can be used to conserve this endemic species by facilitating its mass propagation and transplantation into the natural environment, also replicated in other Himalayan species of the genus *Mahonia* and *Berberis*. Further, study revealed that, *M. jaunsarensis* possess a wide array of alkaloids and have shown good antioxidant and antimutagenic activities which highlights its potential as a vital source of antioxidant polyphenolics.

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**Data Availability** Data sharing is not applicable to this article as all the datasets generated during the current study has been presented in the results.

## Declarations

**Conflict of Interest** Authors declared that there are no known conflicts of interest associated with this publication.

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