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Development of an *In vitro* Propagation Protocol for *Thalictrum foliolosum*: An Endangered Medicinal Plant

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Abstract

The present study has developed a reproducible *in vitro* micropropagation protocol for endemic and endangered *Thalictrum foliolosum* by using the explants of leaf and root segments. *Thalictrum foliolosum* possesses high medicinal properties due to the presence of valuable phenols, flavonoids and alkaloids. The plants are uprooted due to their high medicinal and clinical applications, hence can lead to extinction threat. *In vitro* leaf and root, explant was used for induction of multiple shoots on MS medium supplemented with plant growth regulators like BAP, IBA, NAA and IAA alone or in combination. The best positive response for the formation of shoots from the leaf segment was obtained from MS media supplemented with BAP (1.0 mg/l) + NAA (0.6 mg/l) after 4 weeks and Maximum roots were formed on MS + IBA (0.6 mg/l) within 4-5 weeks. Likewise, the maximum percentage of shooting and rooting from root explant was obtained in MS + BAP (0.8 mg/l) + TDZ (0.4 mg/l) and MS+ IAA (1.0 mg/l) + NAA (2.0 mg/l) after 4 weeks. The well-rooted acclimatized plantlets were successfully transferred to field conditions.

Introduction

Thalictrum foliolosum DC (Ranunculaceae) commonly known as Mamira, Mamiri, and Pilijari, is an herbaceous perennial flowering plant distributed in the temperate Himalayan region (Singh et al. 2018). The root extract of *T. foliolosum* was therapeutically used as a remedy for rheumatism, jaundice, skin disease, and snake bite as well as the best tonic, stomachic, diuretic, abdominal colic pain, antiseptic, toothache and cathartic (Hao et al. 2018, Li et al. 2016). Eye disease can be cured by mixing the root powder of *Boerhavia diffusa* and *Thalictrum foliolosum*.

Biological and abiotic stresses can be combated by secondary metabolites produced by these plants. Quantitative analysis reveals the presence of secondary metabolites such as phenol, flavonoids, terpenes, alkaloids, and saponins which are responsible for antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and anticarcinogen

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properties. Currently *in vitro* cytotoxic activities of root extract of *T. foliolosum* was reported against cancerous cells of the human lungs (Sun et al. 2019).

Various bioactive compounds Berberine, thalrugosidine, jatrorrhizine, palmatine, N, O, O-trimethylsparsiflorine, noroxyhydrastinine, berlambine, thalirugine, thalirugidine thaligosine, thalrugosaminine, thalfoliolosumines A, thalicarpine, and thalfoliolosumines B were observed through GC-MS analysis (Sharma et al. 2020). Due to their high medicinal potential, these bioactive compounds have great demand in the pharmaceutical industrious. *Thalictrum foliolosum* possesses a huge medicinal potential that it can be used to develop a wide variety of pharmaceuticals to solve many health challenges.

In recent years, this plant has been included in Ayurvedic pharmacopeia due to its medicinal value. The pharma industry and local people uproot this plant because of its medicinal importance. Therefore, its population is slowly declining in its natural habitat, and it is considered endangered. Hence, an efficient *in vitro* micropropagation and regeneration procedure is urgently needed for *in vitro* preservation of *T. foliolosum*. Developing a reliable *in vitro* micropropagation method is an excellent method of producing plants quickly as well as preserving threatened medicinal plant germplasm (Semwal et al. 2007, Chen et al. 2016). currently, micropropagted plants are considered the best tool for secondary phytoconstituent production because they provide quick and uninterrupted bioactive compound production (Salih et al. 2021). It was also observed by Riaji et al. (2022) that total phenolic and flavonoid content was enhanced in micropropagted plants as compared to the mother plant.

The culture of many species of the Ranunculaceae family has been reported, such as *Cimcifuga racemosa*, *Coptis teeta*, and *Aconitum heterophyllum*. Although micropropagation of the Thalictrum genus was rarely reported yet *T. flavum* was conserved through *in vitro* seedling germination (Samanani et al. 2002).

Proper sterilized condition, type of plant explant, the genotype of the explant, gelling agent of the medium, and suitable growing condition, are important factors for *in vitro* micropropagation success (Naing et al. 2014, Samala et al. 2017). Auxins and cytokinins are essential components of the *in vitro* regeneration process due to their critical roles in cell cycle regulation and cell division for their organogenic/embryogenic responses. Therefore, the genotype of the plant material significantly impacts the process of regeneration of plantlets (Huang et al. 2018).

By using leaf and root explants, we developed a cost-effective and labor-efficient direct regeneration protocol for *Thalictrum foliolosum*. Different plant growth regulators were evaluated for their effect on shooting and rooting in culture media. Thus we introduced new protocol to the literature in this article, which contributes to the large-scale production of this medicinally important plant.

Materials and Methods

Plant material: The plant required for the experiment was collected from the Hatu peak district of Himachal Pradesh, India at an elevation of 3400m in July month. After collection, the plant was identified from a Botanical Survey of India, Dehradun by comparing it with authentic species present in the herbarium.

The leaf and root explant used for culture were excised from the mother plant and washed running tap water. After that, the explants were cut into 3-4 mm size. Under the sterilized condition explants were immersed in systemic fungicide (Bavistin 0.2%) for about 25 min and then rinsed with distilled water. After fungicide treatment, the explants were surface sterilized with 0.1% mercuric chloride for about five minutes followed by 3-4 repeated washing with sterilized distilled water. The explants were dried on filter papers in a laminar flow air chamber to avoid contamination. Under the sterilized condition, the explants were inoculated on basal MS medium with the help of sterilized forceps.

The basal medium with pH 5.6 was prepared and properly sterilized in an autoclave at 121°C for about 20 min. Filter-sterilized plant growth regulators were added to the sterilized medium in a laminar air flow hood. The test tubes were dispensed with MS media (25 ml in each) and plugged with non-absorbent cotton. After inoculation of explant in culture media, cultures were incubated in dark at 25 ± 2°C under 8 hours of light and 16 hours' dark at an irradiance of 40 µmoles m^{-2} s^{-1}. In the present study, we used four replicate and subculturing was done after 6 weeks. The culture was observed regularly and fumigation was done every week.

The leaf and root explants were inoculated on MS media supplemented with BAP (0.2, 0.4, 0.6, and 1.0 mg/l) and NAA (0.2, 0.3, 0.4, 0.5, 0.6, and 0.8) individually or with a combination of BAP with NAA (0.2, 0.3, 0.4, 0.6, 0.8 and 1.0 mg/l) for the induction of the shoot. Various plant growth regulators are used in different concentrations for the induction and proliferation of multiple shoots. Subculturing was done regularly at an interval of two weeks when a maximum number of shoots were obtained. After that these shoot cultures were transferred to the elongation medium.

Following sufficient development of shoots after maintaining 4-5 weeks of culture, well-elongated shoots from the culture tube were transferred to the rooting media for the induction of roots for 1-2 weeks to get healthy and effective roots. The growth medium contains different concentrations of auxins like IAA and IBA. The percentage of rooting, number of roots, and length of roots were recorded every week.

After rooting the plantlets were removed from the culture media washed with sterilized water to remove the excess culture media and planted in small pots containing soil, sand, and organic fertilizer in a ratio of 1 : 2 : 1. The plants were first kept in a growth room for 2 weeks to prevent them from sudden environmental shock. After proper hardening, these plants were planted to field condition.

The result of the present study was carried out by using two types of explants viz. leaf and root explants of *Thalictrum foliolosum.* There were four replications for each explant and treatment combination. Regeneration responses were observed and recorded after 3 weeks of culture and statistically analyzed by using one-way analysis of variance (ANOVA) followed by comparing mean values using post hoc Duncan's multiple range test.

Results and Discussion

The two types of explants viz. leaf, and root explants were taken for *in vitro* regeneration were responded differently at different concentrations of plant growth regulators.

Firstly, the effect on leaf explants cultured on MS medium with 30% sucrose and supplemented with different growth regulators including BAP, and NAA were recorded after 4 weeks (Table 1 and Fig. 1). The high number of shoots and maximum shoot length were observed when MS media was supplemented with NAA (0.5 mg/l). The highest percentage response for multiplication and proliferation was induced when MS media

Table 1. Effects of BAP and NAA on shoot formation from leaf in *Thalictrum foliolosum.*

Phytohormones (mg/l)	Shoot number (mean ± SE)	Shoot length (mean ± SE)	% response (mean ± SE)
Control	5.7500 ± 0.85391^{abc}	5.0000 ± 0.70711^{b}	17.5000 ± 3.22749^{a}
BAP (0.2)	2.5000 ± 0.64550^{a}	2.7500 ± 0.70711 ^{a}	40.0000 ± 14.14214^{ab}
BAP (0.4)	5.2500 ± 0. .62915^{abc}	5.7500 ±1.31498^{ab}	42.5000 ± 3.22749^{ab}
BAP (0.6)	4.5000 ± 1.04083^{ab}	5.2500 ± 1.18145 ^{b}	55.0000 ± 6.45497^{ab}
BAP (1.0)	5.7500 ± 0. .85391^{abc}	6.6750 ± 0. 26887^{bcd}	42.5000 ± 6.61438 ^{ab}
BAP (0.5) + NAA (0.2)	8.0000 ± 0 .91287^{bcd}	6.6250 ± 0. 71807 ^{bcd}	40.0000 ± 9.12871 ^{ab}
BAP (0.5) + NAA (0.3)	8.0000 ± 1.08012^{bcd}	6.7500 ± 0. 32275 ^{bcd}	47.5000 ± 18.98464 ^{ab}
BAP (1.0) + NAA (0.4)	9.0000 ± 1.08012^{cd}	7.3750 ± 0. 55434 ^{bcde}	52.5000 ± 13.30727 ^{ab}
BAP (1.0) + NAA (0.6)	10.2500 ± 2.01556^{cd}	7.8250 ± 0.63031 ^{cde}	62.5000 ± 10.50793 ^{b}
BAP (1.5) + NAA (0.8)	7.7500 ± 1.31498^{bcd}	8.9500 ± 0.21016 ^{def}	50.0000 ±17.55942 ^{ab}
BAP (1.5) + NAA (1.0)	8.5000 ± 1.70783^{cd}	8.2250 ± 0.62233 ^{cde}	52.5000 ± 13.30727 ^{ab}
NAA (0.2)	10.2500 ± 1.25000^{d}	8.2000 ± 1.60676 ^{cde}	60.0000 ± 6.45497^{b}
NAA (0.3)	8.7500 ± 1.31498^{cd}	8.5250 ± 0.99864 ^{def}	50.0000 ± 6.45497 ^{ab}
NAA (0.4)	10.5000 ± 1.32288^{d}	9.4250 ± 0.21747 ^{ef}	60.2500 ± 12.91882 ^{b}
NAA (0.5)	11.2500 ± 1.10868^{d}	10.8750 ± 0.62500 ^{f}	50.0000 ± 12.46328 ^{ab}
NAA (0.6)	10.7500 ± 0.85391^{d}	9.8750 ± 0.17017 ^{ef}	57.5000 ± 16.13743 ^{ab}
NAA (0.8)	8.0000 ± 1.29099^{bcd}	8.5500 ± 0.80467 ^{def}	55.0000 ± 14.71960 ^{ab}

Data recorded after 4 weeks. Values are mean ± SE of four replicates. Means followed by the different letters were not significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test)

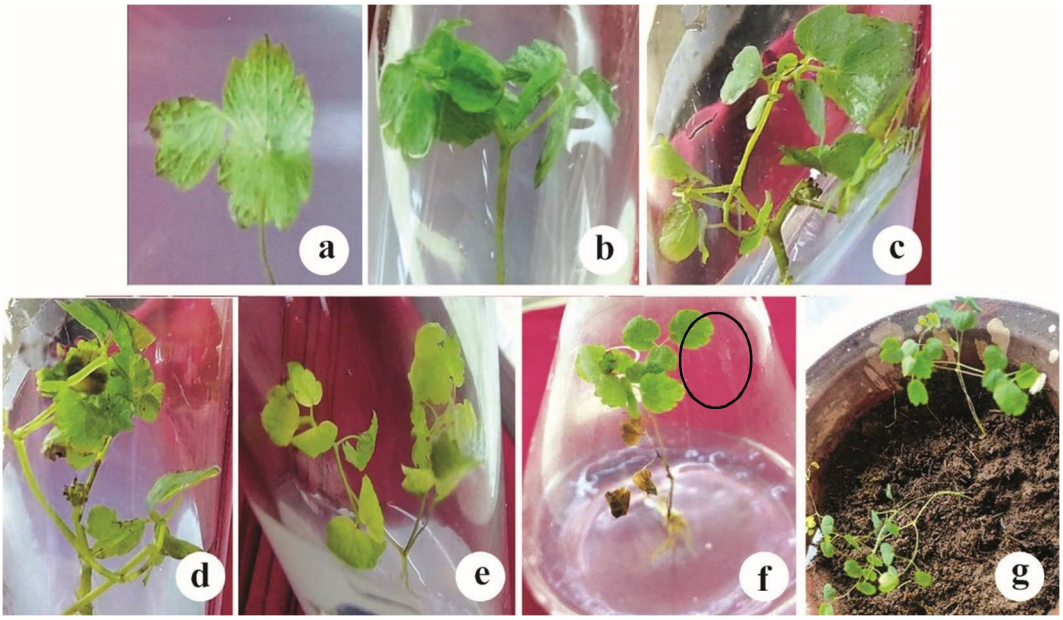

Fig. 1. Development of plantlets from leaf explants: (a) Inoculation of leaf explant (b-d) Shoot induction after 4 weeks of culture (e) Shoot multiplication and elongation after 4-5 weeks in hormone-containing MS, (f) Spontaneous rooting of the regenerated shoot during elongation and multiplication after 4-5 weeks of culture, (e) Healthy rooted acclimatized plantlet ready to transfer in field after 8 weeks**.**

was supplemented with a combination of BAP (1.0 mg/l) + NAA (0.6 mg/l). Many researchers also observed that high BAP concentration helped to increase shoot number and length (Müller and Leyser's 2011). The combined effect of BAP and NAA on increasing shoot length, shoot number, and multiplication was reported for many medicinally important plants like *Homalomena aromatic* (Raomai et al. 2013), and *Psoralea corylifolia* (Anisi and Faisal 2005). The experiment was conducted in 4 replicates and data were recorded after 4 weeks. However, the leaf segment shows less response when grown on basal MS medium.

After the successful shooting, the well-grown shoots were transferred to the rooting media, and the result was observed after 4-5 weeks (Table 2). Different concentration of IBA, and IAA was used to optimize an effective rooting medium for micro-propagated shoots. The maximum no of roots was obtained on MS with IAA (0.4 mg/l) + BAP (0.5 mg/l), the maximum length of roots obtained from MS + IAA (0.4 mg/l) + IBA (0.5 mg/l) and the highest percentage response was obtained with MS + IAA (0.2 mg/l) + BAP (0.4 mg/l). After acclimatization of 8 weeks, these fully prepared plantlets were transferred in filed condition. A number of plants in the Ranunculaceae family, such as *Coptis teeta*, *Thalictrum dalzellii*, and *Aconitum violaceum*, have been studied to determine the effects of

IBA and NAA on root induction efficiency (Rawat et al. 2013, Sharanappa and Rai 2011, Tandon et al. 2007).

Table 2. Effects of auxins on root formation from leaf explant in *Thalictrum foliolosum.*

IAA	IBA	Root number	Root length	% Response
-	-	1.0000 ± 0.40825[a]	1.0250 ± 0.36600[a]	15.0000 ± 2.88675[a]
0.2	-	5.0000 ± 0.91287[b]	5.1750 ± 0.98605[b]	45.0000 ± 6.45497[b]
0.4	-	7.5000 ± 0.64550[bcd]	6.5500 ± 0.56347[cd]	45.0000 ± 6.45497[b]
0.6	-	6.0000 ± 1.08012[bc]	7.5500 ± 0.68496[cd]	50.0000 ± 14.71960[b]
0.8	-	4.5000 ± 1.04083[b]	6.7000 ± 1.09087[cd]	50.0000 ± 4.08248[b]
1.0	-	9.5000 ± 0.64550[d]	8.5500 ± 0.66018[d]	45.0000 ± 11.90238[b]
1.5	-	7.0000 ± 1.58114[bcd]	8.3500 ± 1.30288[d]	47.5000 ± 8.53913[b]
-	0.2	8.7500 ± 0.85391[cd]	6.8750 ± 1.00613[cd]	50.0000 ± 20.41241[b]
-	0.4	6.5000 ± 1.04083[bcd]	8.7300 ± 0.35670[d]	50.0000 ± 8.16497[b]
-	0.6	6.7500 ± 1.10868[bcd]	8.0250 ± 0.61424[d]	50.0000 ± 9.12871[b]
-	0.8	7.5000 ± 0.64550[bcd]	8.1250 ± 0.51539[d]	50.0000 ± 12.90994[b]
-	1.0	9.2500 ± 0.85391[d]	7.0500 ± 0.99205[cd]	35.0000 ± 17.07825[ab]
-	1.5	7.0000 ± 0.91287[bcd]	7.2250 ± 0.78568[cd]	50.0000 ± 16.83251[b]

Data recorded after 4 weeks. Values are mean ± SE of four replicates. Means followed by the different letters were not significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test)

After the successful formation of the complete plantlet from the leaf segment, we conducted the experiment with a second explant viz. root. The root explant was inoculated on MS medium supplemented with different concentrations of growth regulators. The maximum number of shoots was observed on MS + BAP (0.4 mg/l) + TDZ (0.5mg/l), shoot length was obtained on MS+BAP (0.4) + Kn (0.5), and % response was obtained on MS media + BAP (0.2 mg/l) + TDZ (0.4mg/l). The data was recorded after 4 weeks. (Table 3, Figs 2 and 3). The highest number of shoot formations with TDZ (0.5 mg/l) was reported in many other plants like *Artemisia judaica* (Liu et al. 2003), *Thalictrum dalzelli* (Sharanappa et al. 2011), *Scutullaria baicalensis* (Li et al. 2000).

The *in vitro* induced shoots were transferred to the rooting media for root induction. Maximum no of the root was formed with MS + IAA (0.8 mg/l) + IBA (2.0 mg/l). The maximum length was observed when MS media was supplemented with IAA (1 mg/l) + IBA (2.5 mg/l) and after 8 weeks of successful rooting, the plantlets were gradually acclimatized (Table 4). These hardened plants were transferred to natural environmental conditions with 75% survival rate.

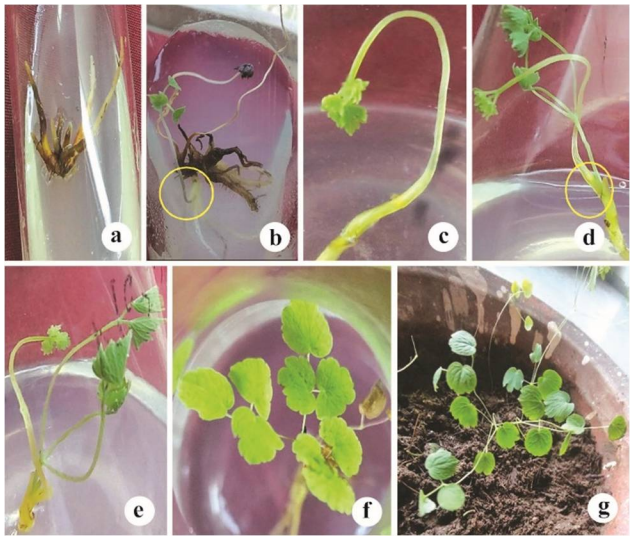

Fig. 2. Development of plantlets from root explants: (a) Inoculation of root explant, (b) Shoot induction after 4 weeks of culture, (c-e) Shoot multiplication and elongation after 5-6 weeks on MS medium + plant growth regulators, (f) Spontaneous rooting of the regenerated shoot during elongation and multiplication after 6-7 weeks of culture, (g) Healthy rooted plantlet ready to transfer in soil after 8 weeks.

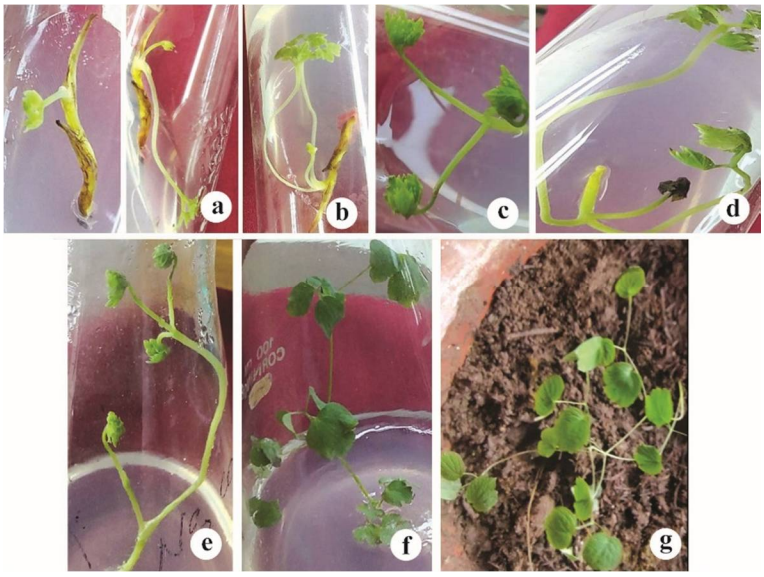

Fig. 3. Development of plantlets from root explants: (a) Shoot induction after 4 weeks of culture, (b-f) Shoot multiplication and elongation after 5-6 weeks on MS medium + BAP + Kn, (g) Spontaneous rooting of the regenerated shoot during elongation and multiplication after 6-7 weeks of culture, (h) Healthy rooted plantlet ready to transfer in the soil after 8 weeks.

Table 3. Formation of shoot from *in vitro* inoculated root explant of *Thalictrum foliolosum* on MS Medium supplemented with growth regulators.

NAA Phyto-hormones (mg/l)	BAP	Kn	TDZ	Shoot Number (Mean ± SE)	Shoot Length (cm) (Mean ± SE)	% Response (Mean ± SE)
Control	-	-	-	-	-	-
0.4	0.2	-	-	3.0000 ± 0.40825^{a}	2.6250 ± 0.31458^{a}	37.5000 ± 3.22749^{a}
0.5	0.4	-	-	4.7500 ± 0.47871^{ab}	3.6500 ± 0.30139^{a}	43.7500 ± 1.25000^{ab}
1.0	0.6	-	-	6.5000 ± 0.50000^{bc}	4.2000 ± 0.28577^{a}	53.7500 ± 2.39357^{bcd}
1.5	0.8	-	-	7.7500 ± 0.47871^{bc}	5.3750 ± 0. .42696^{a}	66.2500 ± 2.39357^{def}
2.0	1.0	-	-	7.5000 ± 0.64550^{bc}	7.0000 ± 0. .40825^{ab}	70.0000 ± 2.88675^{efg}
-	0.2	-	0.4	5.5000 ± 1.19024^{ab}	8.0000 ± 0. .20412^{ab}	81.2500 ± 1.25000^{g}
-	0.4	-	0.5	9.7500 ± 0.62915^{c}	8.9500 ± 0. .10408^{ab}	80.0000 ± 5.40062^{g}
--	0.6	-	1.0	9.2500 ± 1.10868^{c}	9.5000 ± 0. .20412^{ab}	66.2500 ± 8.26009^{def}
-	0.8	-	1.5	7.5000 ± 1.84842^{bc}	9.8750 ± 0. .31458^{ab}	75.0000 ± 2.04124^{fg}
-	1.0		2.0	7.5000 ± 1.04083^{bc}	10.0000 ± 1.35401^{ab}	58.7500 ± 6.57489^{cde}
-	0.2	0.4	-	4.7500 ± 1.54785^{ab}	8.5000 ± 0. .64550^{ab}	51.2500 ± 3.14576^{bc}
-	0.4	0.5	-	5.5000 ± 0.64550^{ab}	15.0000 ± 12.36258^{c}	71.2500 ± 4.26956^{efg}
-	0.6	1.0	-	7.7500 ± 1.88746^{bc}	8.2500 ± 1.79699^{ab}	77.5000 ± 3.22749^{fg}

Data recorded after 4 weeks. Values are mean ± SE of four replicates. Means followed by the different letters were not significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 4. Formation of roots from *in vitro* inoculated root explant of *Thalictrum foliolosum* on MS Medium supplemented with growth regulators.

IAA	BAP	NAA	IBA	Root Number (Mean ± SE)	Root length (cm) (Mean ± SE)	% Response (Mean ± SE)
Control	-	-	-	1.2500 ± 0.25000 ^{a}	2.0000 ± 0.20412 ^{a}	20.0000 ± 4.56435 ^{a}
0.4	0.8	-	-	6.5000 ± 1.04083 ^{bcd}	3.5500 ± 0.64614 ^{b}	40.0000 ± 9.12871 ^{ab}
0.5	1.0	-	-	4.0000 ± 1.08012 ^{b}	5.9000 ± 0.26771 ^{c}	60.0000 ± 11.90238 ^{ab}
0.6	1.5	-	-	6.2500 ± 0.94648 ^{bc}	6.7250 ± 0.38161 ^{cdef}	60.0000 ± 6.45497 ^{ab}
0.8	2.0	-	-	6.7500 ± 0.85391 ^{bcd}	7.0750 ± 0.22867 ^{def}	50.0000 ± 9.12871 ^{ab}
1.0	2.5	-	-	7.0000 ± 0.70711 ^{bcd}	8.2750 ± 0.11087^{gh}	50.0000 ± 12.90994 ^{ab}
0.4	-	0.8	-	6.0000 ± 1.58114 ^{bc}	9.3500 ± 0.19365 ^{i}	50.0000 ± 16.83251 ^{ab}
0.5	-	1.0	-	6.5000 ± 0.95743 ^{bcd}	6.1250 ± 0.57355 ^{cd}	45.0000 ± 9.57427 ^{ab}
0.6	-	1.5	-	7.5000 ± 0.64550 ^{cd}	7.7250 ± 0.39025^{fg}	45.0000 ± 6.45497 ^{ab}
0.8	-	2.0	-	7.7500 ± 0.75000 ^{cd}	8.3750 ± 0.33260 ^{ghi}	53.7500 ± 12.47915 ^{ab}
1.0	-	2.5	-	6.0000 ± 0.70711 ^{bc}	5.9750 ± 0.34248 ^{cd}	63.7500 ± 6.57489 ^{b}
0.4	-	-	0.8	6.7500 ± 1.65202 ^{bcd}	6.2000 ± 0.23805 ^{cd}	52.5000 ± 19.31105 ^{ab}
0.5	-	-	1.0	6.5000 ± 0.64550 ^{bcd}	7.4250 ± 0.04787^{efg}	47.5000 ± 19.31105 ^{ab}
0.6	-	-	1.5	7.5000 ± 1.19024 ^{cd}	6.6000 ± 0.33417 ^{cde}	47.5000 ± 13.76893 ^{ab}
0.8	-	-	2.0	9.5000 ± 0.64550 ^{d}	6.6500 ± 0.46278 ^{cdef}	50.0000 ± 9.12871 ^{ab}
1.0	-	-	2.5	6.5000 ± 0.64550 ^{bcd}	8.9000 ± 0.21213 ^{hi}	50.0000 ± 12.90994 ^{ab}

Data recorded after 4 weeks. Values are mean ± SE of four replicates. Means followed by the different letters were not significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Thalictrum foliolosum is a highly valuable endemic and endangered medicinal plant species in native nature and because of the presence of various valuable alkaloids it has high therapeutic value, that's why there is an urgent need to develop an effective method of their multiplication (Butola et al. 2008). Micropropagation is an efficient means of large-scale production in a very short time with a small amount of maternal material. Therefore, the tissue culture technique is frequently used for the propagation of rare plant species especially when the number and regenerative potential of the mother plant is limited

References

Anis M and **Faisal M** (2005) In vitro regeneration and mass multiplication of *Psoralea corylifolia*-an endangered medicinal plant. Indian J. Biotechnol. **4**: 261-264.

Bedir E, Lata H, Schaneberg B, Khan I A and **Moraes R M** (2003) Micropropagation of *Hydrastis canadensis*: Goldenseal a North American endangered species. *Planta. medica*. **69**(01): 86-88. https://doi.org/ 10.1055/s-2003-37039

Butola JS and **Badola HK** (2008) Threatened Himalayan medicinal plants and their conservation in Himachal Pradesh. *Journal Tropical Medicinal Plants*. **9**(1):125-142

Chen SL, Yu H, Luo HM, Wu Q, Li CF and **Steinmetz A** (2016) Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin Med. **11**: 37. https://doi.org/ 10.1186/s13020-016-0108-7

Hao D C (2018) Ranunculales Medicinal Plants: Biodiversity, Chemodiversity and Pharmacotherapy. Academic Press.

Huang M, Ma X, Zhong Y, Hu Q, Fu M and **Han Y** (2018) Callus induction and plant regeneration of *Spirodela polyrhiza*. Plant Cell, Tissue and Organ Cult. **135**(3): 445-453. https://doi.org/ 10.1007/s11240-018-1477-7

Lata H, Bedir E, Hosick A, Ganzera M, Khan I and **MR Moraes** (2002) In vitro plant regeneration from leaf derived callus of *Cimcifuga racemosa*. Planta Medica. **68**(10): 912-915. https://doi.org/ 10.1055/s-2002-34933

Li Dh, Guo J, Bin W, Zhao N, Wang Kb, Li Jy, Li Zl and **Hua H m** (2016) Two new benzylisoquinoline alkaloids from *Thalictrum foliolosum* and their antioxidant and in vitro antiproliferative properties. Arch. Pharm. Res. **39**(7): 871-877. https://doi.org/ 10.1007/s12272-016-0724-x

Li H, Murch SJ and **PK Saxena** (2000) Thidiazuroninduced de novo shoot organogenesis on seedlings, etiolated hypocotyls and stem segments of Huang-qin. Plant Cell Tissue and Organ Culture. **62**(3): 169-173

Liu CZ, Murch SJ, EL-Demerdash M and **PK Saxena** (2003) Regeneration of the Egyptian medicinal plant *Artemisia judaica* L. Plant Cell Reports. **21**(6): 525-530.

the control of shoot branching. Ann. Bot. **107**(7): 1203-1212. https://doi.org/10.1093/aob/mcr069

Naing AH, Jeon SM, Han JS, Lim SH, Lim KB and **Kim CK** (2014) Factors influencing *in vitro* shoot regeneration from leaf segments of *Chrysanthemum*. Comptes Rendus - Biologies. **337**(6): 383-390. https://doi.org/10.1016/j.crvi.2014.03.005

Phulwaria M, Shekhawat N, Rathore J and **Singh R** (2013) An efficient *in vitro* regeneration and ex vitro rooting of *Ceropegia bulbosa* Roxb.-a threatened and pharmaceutical important plant of Indian Thar Desert. Ind. Crops Prod. **42**: 25-29. https://doi.org/10.1016/j.indcrop.2012.05.013

Rawat JM, Rawat B, Agnihotri RK, Chandra A and **Nautiyal S** (2013) *In vitro* propagation, genetic and secondary metabolite analysis of *Aconitum violaceum* Jacq.: a threatened medicinal herb. Acta Physiol Plant. **35**(8): 2589–2599. https://doi.org/10.1007/s11738-013-1294-x

Riaji L, Chograni H, Rejeb FB, Romdhane MB, Masmoudi AS and **Cherif A** (2022) Efficient *in vitro* regeneration of the endangered species *Artemisia arborescens* L. through direct organogenesis and impact on secondary metabolites production. Hortic. Environ. Biotechnol. **63**: 439-450. https://doi.org/10.1007/s13580-021-00400-8

Samala S, Kongton K, Saisaard K, Nupan B, Thongyai K and **Taweerodjanakarn S** (2017) Effects of plant growth regulators on multiple shoots regeneration of *in vitro Chrysanthemum morifolium Ramat*. Acta Hortic. **1167**(1): 143-148. https://doi.org/10.17660/ActaHortic. 2017. 1167.22

Samanani N, Park S and **Facchini PJ** (2002) *In vitro* regeneration and genetic transformation of the berberine producing plant, *Thalictrum flavum ssp. glaucum*. Physiologia Plantarum. **116**(1): 79-86. https://doi.org/10.1034/j.1399-3054.2002.1160110.x

Salih AM, Al-Qurainy F, Khan S, Tarroum M, Nadeem M, Shaikhaldein HO, Aladallah NM, Alansi S and **Alshameri A** (2021) Mass propagation of *Juniperus procera* Hoechst. Ex. Endl. From seedling and screening of bioactive compounds in shoot and callus extract. BMC Plant Biol. **21**(1): 1-13.https://doi.org/10.1186/s12870-021-02946-2

Semwal D, Saradhi PP, Nautiyal B and **Bhatt A** (2007) Current status, distribution and conservation of rare and endangered medicinal plants of Kedarnath wildlife sanctuary, Central Himalayas, India. Curr Sci India. **92**: 1733-1738

Sharanappa P and **Rai VR** (2011) Micropropagation of *Thalictrum dalzellii* Hook through rhizome buds. J Phytol. **3**(5): 51–55

Sharma N, Kumar V, Chopra MP, Sourirajan A, Dev K and **El-Shazly M** (2020) *Thalictrum foliolosum*: A lesser unexplored medicinal herb from the Himalayan region as a source of valuable benzyl isoquinoline alkaloids. *Journal of ethnopharmacology*. ***255***: 112736. https://doi.org/10.1016/j.jep.2020.112736

Singh H, Srivastava A, Kumar A, Sundaresan V and **Husain T** (2018) *Thalictrum nainitalense* (Ranunculaceae), a new species from the Uttarakhand Himalaya, India. Folia Geobot. **53**(4): 449-455. https://doi.org/10.1007/s12224-018-9326-3

Sun N and **Han Y** (2019) Cytotoxic isoquinoline alkaloids from the roots of *Thalictrum foliolosum*. J. Asian Nat. Prod. Res. **23**(1): 1-8. https://doi.org/10.1080/10286020.2019.1694515

Tandon P, Rathore TS and **Kumaria S** (2007) Micropropagation of *Coptis teeta* Wall. -threatened medicinal plant of Arunachal Pradesh, India. Indian J Biotechnol. **6**: 280-282

Tandon P and **Rathore TR** (1992) Regeneration of plantlets from hypocotyls derived callus of *Coptis teeta*. Plant Cell Tissue and Organ Culture. **28**(1): 115-117

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