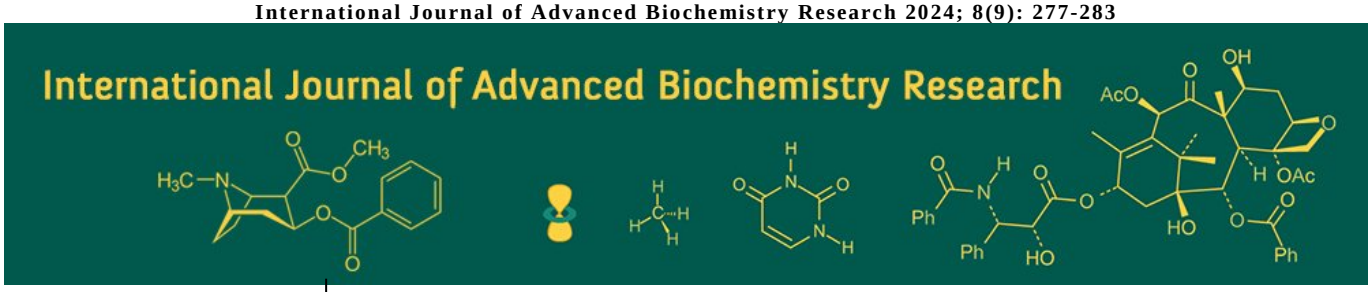

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In vitro and *ex vitro* rhizogenesis studies in Sarpagandha (*Rauwolfia Serpentina* L.)

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Abstract
Rauwolfia serpentina L., is a widely used medicinal woody herb species listed as endangered due to overharvesting by the pharmaceutical sector. This misuse has led to a reduction in the species population, placing it at risk. Thus, the development of nonconventional methods for sarpagandha conservation is vitally needed. The purpose of this experiment was to assess the rooting efficiency of *in vitro* grown shoots using *in vitro* and *ex vitro* rooting method. A rooting efficiency of 96.67% was achieved *in vitro* propagated shoots, with an average of 5.17 roots per shoot, when cultured on one-fourth strength Murashige and Skoog (MS) medium augmented with 2.0 mg/l indole-3-butyric acid (IBA). The highest *ex vitro* rooting was reported on soilrite and cocopeat (1:3) when the shoots were dipped in 200 mg/l of indole-3 butyric acid for 30 minutes. Plantlets that were rooted both *in vitro* and *ex vitro* showed 60 percent and 70 percent survival rates after being successfully hardened in the greenhouse.

Keywords: *Ex vitro* rooting, *in vitro* rooting, murashige and skoog medium, Sarpagandha

Introduction
Rauwolfia serpentina is another most important endangered medicinal plant species in the Apocynaceae family, also called by the common name sarpagandha. Owing to the high concentration of secondary metabolites, which are mostly concentrated in the roots and rhizomes, leaves, and juice have gained interest from practitioners of the indigenous medical system (Mittal *et al*., 2012) [16]. The roots of sarpagandha are used for treating anxiety, dyspepsia, hypertension, insomnia, and various central nervous system disorders. Along with these additional uses, it can be an antithelmintic, improve uterine contractions, eliminate corneal opacities, and treat snake venom (Jain *et al*., 2003 and Panwar *et al*., 2011) [8, 21].
This species has rapidly declined due to overexploitation by the pharmaceutical sector, poor seed germination and viability (10%), a low rate of vegetative propagation, and habitat degradation. Thus, it has been listed as endangered by Red Data Book. Sarpagandha seed germination varies greatly, even when heavy seeds are selected for planting. Sarpagandha is commercially cultivated through seed propagation. However, the main challenges in propagating sarpagandha seeds are seed dormancy and the absence of a viable embryo. Farmers are being forced to employ cuttings for planting material multiplication due to the issue of low germination rate (Pant and Joshi, 2008) [20]. Thus the best technique for increasing the quantity of planting material available at any given period of the year is micropropagation, it has been discovered. The current studies aim to determine the optimal culture medium for maximum shoot regeneration by analyzing the impact of explant size on various basal mediums. *In vitro*, and *ex vitro* rooting methods were used to assess the *in vitro* regenerated shoots ability to root under the effect of varying phytohormone concentrations to produce plantlets quickly. Finally hardening of rooted plantlets to open the field for survival, will also be studied.

Materials and Methods
Source and surface disinfection of nodal explants
The Nagarjuna Medicinal and Aromatic Plant Garden, Dr. PDKV, Akola was the source of the nodal explants used in the trial.

Nodal segments are harvested from mother plants by cutting the plant portions with sterile instruments, such as secateurs, knives, or forceps. The nodal segments were subjected to a continuous 10 minute washing cycle with running tap water. They were then exposed to 70% (volume/volume) ethanol solution for 30 seconds, followed by four rinses with distilled water. Afterwards, the nodal explants underwent a 1 minute treatment with 2 drops of Tween 20, then treated with the fungicide Bavistin 0.1% for 30 minutes and the antibiotic streptomycin 1% for 15 minutes at room temperature. Finally, four more times, the distilled water was used to eliminate the remaining sterilants. After treating the explants for 10 minutes with 1.0% NaOCl and 5 minutes with 0.1% HgCl2 in a laminar airflow, the plants were rinsed again to get rid of the remaining NaOCl and HgCl2, and then they were placed in a cooled antioxidant solution for a duration of 5 to 10 minutes to prevent phenolic release.

Culture medium and conditions

The full strength Murashige and Skoog (1962) [17] and Lloyd and McCown (1981) [12] medium, along with plant growth regulators, were used for the *in vitro* regeneration of sarpagandha. The culture medium, with its pH set to 5.8 and was subjected to stream sterilized by autoclaving at 121 °C and 15 psi pressure for a duration of 15 minutes. The culture bottles filled with media were then exposed to continuous light for 16 hours, then placed in darkness for 8 hours.

Induction and shoot proliferation

The nodal segments are selected from the basal and middle portions of the secondary branches. Cut the explants into three different diameters (5, 7, and 10 mm) with a scalpel. After being divided into segments, inoculate the medium using forceps under a laminar air flow unit. The optimization of plant growth regulators for shoot initiation was achieved using MS and WPM media as the basal medium, supplemented with cytokinin BAP at 2 mg/l and varying concentrations of auxin NAA (0.3, 0.5, and 0.7 mg/l). At intervals of seven days, shoot clumps containing mother explants were moved to a fresh culture medium to multiply cultures containing initiated shoots.

***In vitro* rooting of shoots**

In order to cultivate *in vitro* roots, regenerated shoots (4-6 cm long) were saperated and moved to one-fourth strength MS medium containing varying concentrations of rooting hormones IAA and IBA (0.5 to 4.0 mg/l). Then, this cultures were maintained under LED light for a period of three weeks.

***Ex vitro* rooting of shoots**

To facilitate *ex vitro* rooting, 4-6 cm long *in vitro* regenerated shoots were cut and subjected to treatment of auxins (IBA and IAA) at varying concentrations (ranging from 50 to 500 mg/l) for 30 minutes. Afterwards, the shoots treated with auxin were transferred to a autoclaved soilrite and cocopeat (1:3) hardening media dampened with a one-fourth aqueous MS macro-basal salt solution. The treated shoots were moved to a greenhouse under natural light, where they were kept moist and protected from desiccation by being covered with glass jar for ten to fifteen days. Every day, the glass jars were removed for one to two hours to allow the plantlets to become acclimated to the external environment. After a 5 week period, data were recorded on the rooting and survival percentages, the average roots number per shoot and their length.

Hardening and acclimatization

Initiating their roots for 30 days, the micropropagated plantlets were transferred to a greenhouse under partial natural conditions, where both plantlets with roots grown *in vitro* and those grown *ex vitro* underwent hardening process. Following that, they were carefully moved to nursery polybags, which were filled with a 1:1:1 ratio of garden soil, organic manure, and red soil. once rooted plantlets fully hardened, transfer to the field.

Statistical analysis

In the experiment, three replications were conducted with thirty explants used for each treatment. The experiment was designed using both a Factorial Completely Randomized Design (FCRD) and a Completely Randomized Design (CRD). The statistical significance of the treatment means was analysed by calculating the critical difference at 1%.

Results and Discussion

Shoot initiation and multiplication of shoots on MS and WPM medium

The effectiveness of micropropagation is largely contingent upon the choice of an appropriate plant tissue and its size, which serve as the primary material for the procedure. Our study obtained promising results on MS medium, compared to WPM medium, when augmented with 0.7 mg/l NAA and 2.0 mg/l BAP. The MS medium achieved 90% shooting with an average of 3.36 shoots per explant initiated after the 1st, 2nd, and 3rd subcultures with 5.15, 8.14, and 10.43 shoots per culture, respectively, which was found to be significant and superior. Also study found that 10 mm long nodal explants were significantly superior to 5 mm and 7 mm long nodal explants, showing 74.17% shooting, an average of 3.04 shoots and 4.10, and 7.49 shoots per culture in 1st and 3rd subcultures on MS medium compared to WPM medium with 59.17% shooting (Table 1, Figure 1). However, the frequency of shoot formation was significantly increased by the addition of NAA at the optimal concentration of BAP consistent with previous studies showing that NAA promotes the proliferation and elongation of shoots in species *Hemidesmus indicus* (Sreekumar *et al*.,2000) [31], *Holostemma ada-kodien* (Martin, 2002) [14] and *Mucuna pruriens* (Faisal *et al*., 2006) [6]. Mallick *et al*. (2011) [13] reported direct regeneration (96%) in MS medium augmented with 0.2 mg/l NAA and 1.5 mg/l BAP, emphasizing the need for high cytokinin and low auxin levels. In contrast, Alatar *et al.*, (2012) [1] achieved the highest frequency of shoot regeneration (90%) and the maximum number of shoots (45.4 ± 3.2 per explant) with an average shoot length of (4.7 ± 0.21 cm) on WPM medium supplemented with 5.0 µM BAP and 1.0 µM NAA. Pandey (2007) [19] found MS medium supplemented with 1 mg/l NAA and 2 mg/l BAP to be optimal for shoot proliferation, while Roja and Heble (1996) [27] obtained multiple shoots from axillary buds using 1 mg/l BAP and 0.1 mg/l NAA. Pandey *et al*., (2007) [19] achieved the best shoot proliferation using 1.5 cm long shoots supplemented with 1 mg/l NAA and 2 mg/l BAP on MS medium.

***In vitro* rooting of shoots on one-fourth MS medium**

In our study, we observed that root induction was significantly higher in a one-fourth strength MS medium supplemented with 2.00 mg/l IBA, resulting in 96.67% root induction, with an average of 21.99 days, 5.17 roots per shoot, and the highest average root length of 5.38 cm (Table 2). However, IAA was found less effective for root induction, with a rooting percent of 90%, an average

number of 23.30 days, and 4.37 roots per shoot. The roots were long, pale white, and robust with root hairs (Figure 2). The present results were in accordance with results reported by Shekhawat and Manokari, (2015) [29] who obtained 4.4 ± 0.35 roots on a one-fourth strength MS medium enhanced with 2.0 mg/l indole-3 butyric acid (IBA). Similarly, Rani *et al*., (2014) [25] reported 88% of *in vitro* root induction on half strength MS medium with 3 mg/l IBA. Bhatia *et al*., (2002) [4] found that inducing roots was most successful with IBA when compared to indole-3-acetic acid (IAA). According to van der Krieken *et al*., (1993) [32], IBA was absorbed four times faster than IAA. Furthermore, previous research by Kour *et al*. (2014) [10] supports our *in vitro* rooting results, although they observed the greatest response using a half-strength MS medium supplemented with 0.5 mg/l IBA.

***Ex vitro* rooting of shoots on one-fourth MS medium**

The study found that excised shoots rooted *ex vitro* with a 200 mg/l IBA concentration achieved a 70% rooting success rate, which was notably higher than other treatments. These shoots developed an average of 4.29 roots per shoot and an average root length of 4.55 cm (Table 3). In comparison, 300 mg/l of IAA resulted in 63.33% rooting, an average number of roots per shoot of 3.83, average root length of 3.56 cm, which was significantly superior over other treatments. The process of transferring micro propagated plantlets from *in vitro* to *ex vitro* conditions is critical because the plantlets underdeveloped cuticle and stomata cause them to wilt in low humidity, fungal attack, and high light intensities. According to Annapurna and Rathore (2010) [2], *ex vitro* plants exhibit a greater proportion of survival frequency and rapid adaptability to their natural environment. In accordance with the present findings, Shekhawat and Manokari (2015) [29] reported IBA effectively induces roots in *Artemisia absinthium* L. in *ex vitro*. In this study, a higher percentage of shoots were rooted when treated with 200 mg/l of IBA, with a maximum average of 7.3±0.40 roots per shoot and an average root length of 6.84±0.24 cm. Patil and Jayanthi (1997) [22] reported successful *ex vitro* rooting of sarpagandha by supplementing the potting mixture with 100 mg/l IBA. According to studies by Mishra *et al*. (2010) [15], Kataria and Shekhawat (2005) [29], and Pant and Joshi (2008) [20], *In vitro* grown shoots treated with NAA showed 100%, 98%, and 86.6% *ex vitro* rooting respectively. Like the sarpagandha plants natural root system, the *ex vitro* developed plantlets possessed thick roots at the base of their micro cuttings that lacked callus (Figure 3, 4). Moreover, roots grown *in vitro* have been noted to be thin, fragile, and prone to breakage during handling, unlike roots developed through the *ex vitro* method (Figure 2).

Hardening and Acclimatization

Ex vitro rooting is a one of the cost-effective method of micropropagation since it simultaneously roots and hardens the plants (Nemeth, 1986) [18]. In recent years, many tissue culturists have come to emphasize *ex vitro* rooting over *in vitro* rooting when growing plants on a commercial scale because it shortens the micropropagation steps, labor, time and saves costs. In the current study, both *in vitro* and *ex vitro* rooting was continued for 4 weeks. After this period, the *in vitro* rooted shoots were taken out of the culture vessels after this time, and the agar medium was washed from the roots using running tap water. The cleaned shoots were then placed into protrays with a hardening media soilrite and cocopeat (1:3 and 1:1). These shoots underwent a hardening process in the greenhouse for 3 weeks (Figure 5).

In the hardening process for tissue-cultured plants, a 1:3 soilrite and cocopeat mixture proved best, achieving a maximum survival rate of 53.33%. In this treatment, 210 plants were transferred, of which 112 survived while a 1:1 soilrite and cocopeat mixture resulted in lower survival rate of 19.04%, with only 40 out of 210 plants surviving. After 5 weeks of hardening, the survival percentages of the plantlets were recorded 60% for *in vitro* rooted plantlets and 70% for *ex vitro* rooted plantlets. In both treatments, an initial number of 30 shoots were utilized. Thus, the transfer of plantlets from the culture jar into the soil requires a careful stepwise procedure. Due to abrupt changes in the environment, micropropagated plantlets exhibit low survival or reduced growth rate when transferred from *in vitro* to *ex vitro* conditions, such as a greenhouse or field (Eliasson *et al*., 1994 and Pospisilova *et al*., 1999) [5, 23]. The plants experience morphological and physiological changes during acclimatization, which allows them to establish typical terrestrial plant water control. This process takes place over the course of two to three weeks as the humidity is gradually reduced (Grout and Aston, 1977) [7]. *In vitro* cultures create unique conditions that often lead to the development of plantlets with atypical morphology, anatomy, and physiology. When these plantlets, cultivated under *in vitro* conditions, are moved to a less humid external environment, they frequently experience desiccation, water loss, and eventually death (Selvapandiyan *et al*., 1988) [35].

To overcome these abnormalities, an acclimatization period of two to three weeks is required. One of the key elements in determining the plants survival rate during the acclimatization process is the type of potting mixture used to acclimate them in an *ex vitro* condition. Soilrite, exhibits superior water retention capabilities, due to its greater porosity relative to vermiculite and garden soil.This enhanced water-holding capacity facilitates better growth of tender roots in micropropagated plants during the hardening phase. Prakasha *et al*. (2017) [24] reported similar findings, concluded that a combination of soilrite and cocopeat in a 1:3 ratio produced best results one week after planting. Wahurwagh *et al*. (2022) [34] observed that the most effective results were achieved when the 1:3 soilrite and cocopeat mixture was watered daily. Conversely, Singh *et al*. (2009) [30] found that the hardening process for *in vitro* rooted plantlets was unsuccessful. At present, there is a lack of data in the existing literature regarding the survival rates of *in vitro* plantlets during the hardening process. However, Rani *et al*. (2014) [25] reported a survival rate of 70% for hardened plants transferred to the field. Similarly, Rashmi and Trivedi (2016) [26] observed a survival rate of 77% for these plants. Both studies noted that the transplanted plants showed no noticeable differences in morphology or growth characteristics relative to the mother plants. Plantlets established in *ex vitro* underwent a gradual adaptation to their natural environment during the rooting process, according to research by Benmahioul *et al*., (2012) [3]. Yan *et al*. (2010) [3, 35] demonstrated that the *ex vitro* rooting technique proved superior to the *in vitro* method, as plantlets generated through *ex vitro* rooting showed significantly greater root length, higher rooting efficiency, and improved transplant survival compared to those developed *in vitro*.

Additionally, these plantlets produced lateral roots without callus formation at the base of micro cuttings, closely resembling a natural root system. *Ex vitro* rooted plantlets, on the other hand, showed superior growth with morphologically robust and well-developed root systems that support plantlets in field conditions after transplantation. Liu and Li, (2001) [11] noticed a better survival rate of plantlets through *ex vitro* than *in vitro*. *Ex vitro* rooting is both cost-effective and time-efficient, and it simplifies the process by eliminating the need for sterile conditions during rooting (Pospisilova *et al*., 1999; Yan *et al*., 2010) [23, 35]. Additionally, plantlets rooted through these methods exhibit enhanced resilience to environmental stresses during the hardening phase (Vengadesan and Pijut, 2009) [33]. No observable morphological differences were noted between the hardened plants and the donor plants, in contrast to significant changes observed in *in vitro*-raised plants during the acclimatization process.

Table 1: Effect of NAA and BAP on shoot multiplication from nodal explants of sarpagandha on MS and WPM medium

Treatments	Shooting %		Days to shoot initiation		Number of shoots initiated		Number of multiples initiated after subculture 1		Number of multiples initiated after subculture 2		Number of multiples initiated after subculture 3	
	MS	WPM	MS	WPM	MS	WPM	MS	WPM	MS	WPM	MS	WPM
Factor A : Medium combination												
P1. (Control)	47.78 (43.66)	36.67 (36.14)*	17.01	21.00	1.75	1.44	2.31	2.24	3.24	3.31	4.67	4.24
P2. (0.3 mg/l NAA + 2.0 mg/l BAP)	60.00 (51.14)	44.44 (41.47)	14.37	16.30	2.13	1.96	2.55	2.33	3.33	3.51	4.83	4.61
P3. (0.5 mg/l NAA + 2.0 mg/l BAP)	74.44 (59.74)	61.11 (51.67)	10.68	15.28	2.21	2.02	3.06	3.13	3.91	4.34	5.39	5.28
P4. (0.7 mg/l NAA + 2.0 mg/l BAP)	90.00 (73.86)	77.78 (62.12)	11.04	12.75	3.36	3.08	5.15	4.63	8.14	5.97	10.43	7.54
SE (m)±	1.23	0.74	0.40	0.26	0.15	0.09	0.20	0.09	0.27	0.10	0.41	0.20
CD (P= 0.01)	4.85	2.95	1.58	1.03	0.60	0.34	0.81	0.35	1.08	0.41	1.63	0.80
Factor B : Size of explant												
A1. (5mm)	65.00 (54.74)	52.50 (45.99)	15.50	19.02	1.81	1.89	2.74	2.77	3.92	3.97	5.78	4.92
A2. (7mm)	65.00 (56.77)	53.33 (47.18)	13.74	14.43	2.24	2.10	2.96	2.99	4.56	4.38	5.73	5.34
A3. (10mm)	74.17 (59.79)	59.17 (50.39)	10.58	15.56	3.04	2.38	4.10	3.48	5.49	4.51	7.49	6.00
SE (m)±	1.06	0.65	0.35	0.23	0.13	0.07	0.18	0.08	0.24	0.09	0.36	0.18
CD (P= 0.01)	4.20	2.55	1.37	0.89	0.52	0.29	0.70	0.31	0.93	0.36	1.41	0.69
Interaction A × B : (Medium combination × Size of explant)												
P1A1	30.00 (33.21)	10.00 (18.43)	20.67	29.67	1.22	1.00	1.67	1.33	2.11	2.33	3.01	3.17
P1A2	50.00 (45.00)	40.00 (39.23)	15.80	14.67	1.93	1.50	2.00	2.53	3.05	3.42	4.45	4.44
P1A3	63.33 (52.78)	60.00 (50.77)	14.56	18.67	2.09	1.83	3.26	2.87	4.57	4.19	6.53	5.11
P2A1	60.00 (50.77)	46.67 (43.08)	16.70	20.53	1.28	1.80	1.86	2.17	2.63	3.50	4.24	4.36
P2A2	40.00 (39.23)	20.00 (26.57)	14.67	14.00	1.58	1.92	1.95	2.00	2.74	3.00	3.60	4.00
P2A3	80.00 (63.43)	66.67 (54.78)	11.75	14.38	3.54	2.15	3.83	2.82	4.62	4.04	6.66	5.48
P3A1	80.00 (63.43)	73.33 (59.00)	10.58	12.62	1.96	2.28	3.31	3.18	3.14	4.32	5.76	5.03
P3A2	73.33 (59.00)	70.00 (56.79)	14.74	15.48	1.83	1.86	1.82	2.47	3.28	3.97	3.14	4.80
P3A3	70.00 (56.79)	40.00 (39.23)	6.71	17.75	2.86	1.92	4.04	3.73	5.33	4.73	7.27	5.99
P4A1	90.00 (71.57)	80.00 (63.43)	14.04	13.25	2.78	2.50	4.12	4.40	7.82	5.72	10.10	7.10
P4A2	96.67 (83.86)	83.33 (66.14)	9.77	13.58	3.63	3.11	6.06	4.97	9.17	7.12	11.70	8.11
P4A3	83.33 (66.14)	70.00 (56.79)	9.32	11.43	3.68	3.62	5.25	4.50	7.45	5.08	9.49	7.42
SE (m)±	2.12	1.29	0.69	0.45	0.26	0.15	0.35	0.15	0.47	0.18	0.71	0.35
CD (P= 0.01)	8.39	5.10	2.74	1.79	1.03	0.59	1.40	0.61	1.87	0.72	2.83	1.39

*'' Arc sin transformed

Table 2: Effect of IBA and IAA on *in vitro* rooting of sarpagandha shoots on one-fourth MS medium

Treatment number	Medium composition 1/4th MS + PGR (IBA mg/l and IAA mg/l)	% Rooting		Number of days for rooting		Number of roots/ shoot		Root length (cm)	
		IBA	IAA	IBA	IAA	IBA	IAA	IBA	IAA
T_1	0	0.00 (0.00)*	0.00 (0.00)*	0.00	0.00	0.00	0.00	0.00	0.00
T_2	0.5	60.00(50.77)	46.67(43.08)	27.17	27.43	1.28	1.27	1.83	1.37
T_3	1.0	73.33(59.00)	60.00(50.77)	25.86	27.06	2.19	1.83	2.56	2.10
T_4	1.5	90.00(71.57)	66.67(54.78)	23.69	26.81	3.33	3.81	3.10	3.05
T_5	2.0	96.67(83.86)	90.00(71.57)	21.99	23.30	5.17	4.37	5.38	3.11
T_6	3.0	80.00(63.43)	83.33(66.14)	24.00	25.56	4.22	3.26	4.86	4.46
T_7	4.0	70.00(56.79)	70.00(56.79)	25.38	26.24	2.76	2.29	2.84	2.51
	SE (m) ±	2.47	1.47	0.28	0.35	0.12	0.11	0.11	0.13
	CD 1%	10.39	6.18	1.18	1.49	0.49	0.47	0.47	0.55

*” Arc sin transformed

Table 3: Effect of IBA and IAA on *ex vitro* rooting of *in vitro* regenerated shoots of sarpagandha

Treatment number	Auxins (IBA and IAA) concentration (mg/l)	% Rooting		Number of roots/ shoot		Root length (cm)	
		IBA	IAA	IBA	IAA	IBA	IAA
T_1	Control	0.00(0.00)*	0.00(0.00)*	0.00	0.00	0.00	0.00
T_2	50	23.33(28.78)	33.33(35.22)	1.69	1.07	1.77	1.16
T_3	100	30.00(33.21)	30.00(33.21)	1.58	1.50	1.95	1.42
T_4	200	70.00(56.79)	46.67(43.08)	4.29	2.23	4.55	2.51
T_5	300	56.67(48.85)	63.33(52.78)	3.10	3.83	3.46	3.56
T_6	400	53.33(46.92)	40.00(39.23)	2.95	2.67	2.26	2.93
T_7	500	40.00(39.23)	36.67(37.22)	2.40	1.37	2.77	2.17
	SE (m) ±	1.33	1.50	0.10	0.10	0.09	0.06
	CD 1%	5.58	6.32	0.44	0.41	0.38	0.24

*” Arc sin transformed

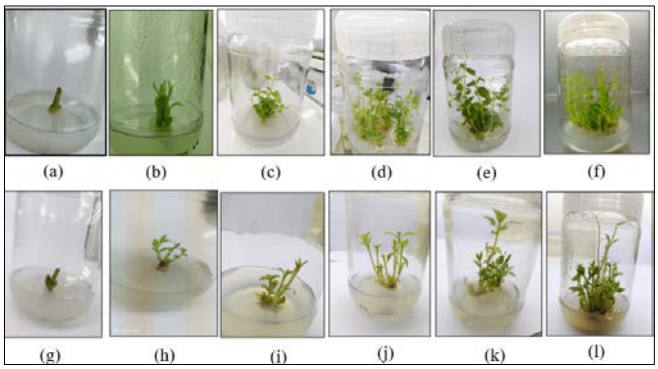

Fig 1: (a) Inoculated nodal explant of size 10 mm on MS medium. (b) Shoot with explant after 1 week on MS medium. (c), (d), (e) and (f) *in vitro* multiplication of shoots on MS medium with NAA and BAP. (g) Inoculated nodal explant of size 10 mm on WPM medium. (h) Shoot with explant after 1 week on WPM medium. (i), (j), (k) and (l) *in vitro* multiplication of shoots on WPM medium with NAA and BAP.

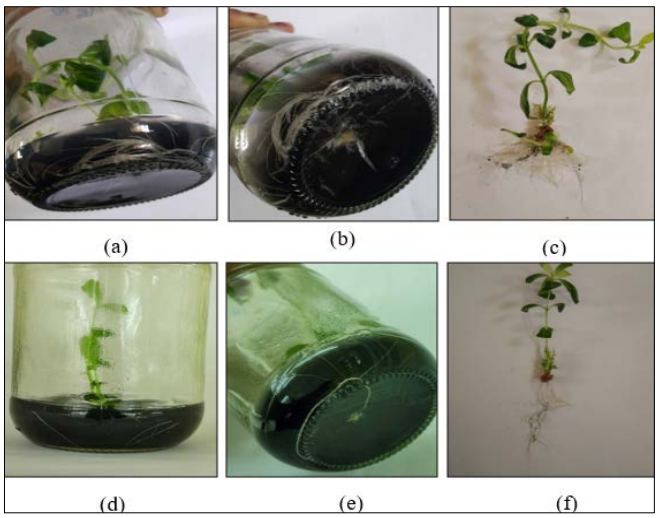

Fig 2: (a) *In vitro* root induction after 1 week from the cut end of the shoots with IBA (b) *In vitro* root induction after 2 weeks from the cut end of the shoots with IBA (c) *In vitro* rooted plantlet from the cut end of the shoots with IBA. (d) *In vitro* root induction after 1 week from the cut end of the shoots with IAA (e) *In vitro* root induction after 2 weeks from the cut end of the shoots with IAA (f) *In vitro* rooted plantlet from the cut end of the shoots with IAA.

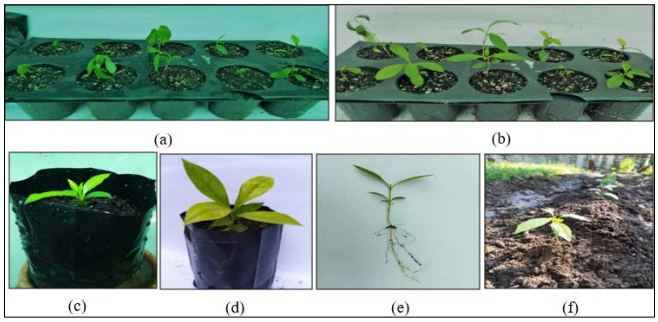

Fig 3: (a) Shootlets treated with 200 mg/l IBA. (b) Plantlet after 5 weeks. (c) and (d) 1 ½ to 2 month old plantlet maintained under greenhouse condition. (e) 4 week-old *ex vitro* rooted plantlet. (f) Field transferred plant of sarpagandha.

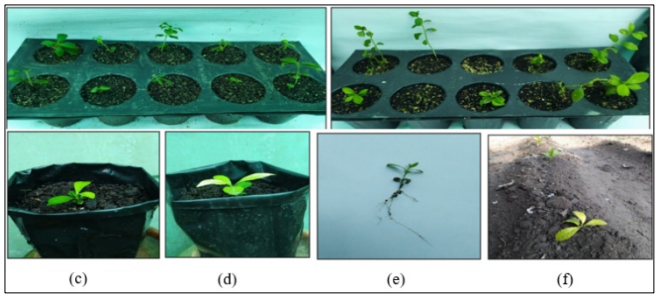

Fig 4: (a) Shootlets treated with 300 mg/l IAA. (b) Plantlet after 5 weeks. (c) and (d) 1 ½ to 2 month old plantlet maintained under greenhouse condition. (e) 4 week-old *ex vitro* rooted plantlet. (f) Field transferred plant of sarpagandha.

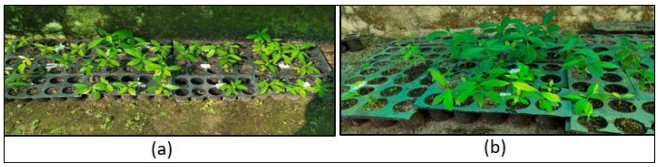

Fig 5: (a) Hardening of *in vitro* rooted plantlets in 1:3 soilrite and cocopeat mixture under greenhouse. (b) Hardening of *ex vitro* rooted plantlets in 1:3 soilrite and cocopeat mixture under greenhouse.

Conclusion

In conclusion, *ex vitro* rooting eliminates the need for sterilization and combines the rooting and transplanting processes. This results in a significant reduction in the expense of tissue culture, as well as time, energy, labor, and resource savings that increase the percentage of plantlets that survive during hardening and field transfer. An *in vitro* and *ex vitro* rhizogenesis protocol can be used for mass propagation of endangered medicinal sarpagandha species, *Rauwolfia serpentina*. These protocols facilitate the large-scale cultivation of this valuable plant by promoting successful root development both within controlled environments and in natural settings. The data could substantially aid in fulfilling the market demand for this multifunctional medicinal shrub and in conserving this genotype using biotechnological approaches.

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Declaration

Conflict of interest: The authors assert that they have no competing interests.

Ethical approval: The submitted manuscript is original and has not been previously published in any format or language.

References

1. Alatar AA, Faisal M, Hegazy AK, Hend AA. High frequency shoots regeneration and plant establishment of *Rauvolfia serpentina*: An endangered medicinal plant. Journal of Medicinal Plants Research. 2012;6(17):3324-3329.
2. Annapurna D, Rathore TS. Direct adventitious shoot induction and plant regeneration of *Embelia ribes* Burm F. Plant Cell, Tissue and Organ Culture. 2010;101(3):269–277.
3. Benmahioul B, Dorion N, Harche MK, Daguin F. Micropropagation and ex vitro rooting of pistachio (*Pistacia vera*). Springer Science+ Business Media. 2012;353-358. DOI:10.1007/s11240-011-0040-6.
4. Bhatia NP, Bhatia P, Ashwath N. Ex vitro rooting of micropropagated shoots of *Stackhousia tryonii*. Biologia Plantarum. 2002;45(3):441-444.
5. Eliasson MK, Beyl CA, Barker PA. *In vitro* responses and acclimatization of *Prunus serotina* with paclobutrazol. Journal of Plant Growth Regulation. 1994;13:137-142.
6. Faisal M, Siddique I, Anis M. *In vitro* rapid regeneration of plantlets from nodal explants of *Mucuna pruriens* - a valuable medicinal plant. Annals of Applied Biology. 2006;148:1-6.
7. Grout B, Aston MJ. Transplanting of cauliflower plants regenerated from meristem culture. Horticultural Science. 1997;17:1-7.
8. Jain V, Singh D, Saraf S, Saraf S. *In vitro* micropropagation of *Rauwolfia serpentina* through

multiple shoot generation. Ancient Science of Life. 2003;23(1):44.
9. Kataria V, Sekhawat NS. Cloning of *Rauvolfia serpentina* - an endangered medicinal plant. Journal of Sustainable Forestry. 2005;20(1):53-65.
10. Kour B, Kour G, Kaul S, Dhar MK. *In vitro* mass multiplication and assessment of genetic stability of *in vitro* raised *Artemisia absinthium* L. plants using ISSR and SSAP molecular markers. Advances in Botany; c2014 .p. 2014.
ID 727020.
11. Liu Z, Li Z. Micropropagation of *Camptotheca acuminata* Decaisne from axillary buds, shoot tips, and seed embryos in a tissue culture system. *In vitro* Cellular & Developmental Biology - Plant. 2001;37:84–88.
12. Lloyd G, McCown B. Commercially-feasible micropropagation of Mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. The International Plant Propagators Society. 1981;30:421-427.
13. Mallick SR, Jena RC, Samal SC. Rapid *in vitro* multiplication of an endangered medicinal plant sarpagandha (*Rauwolfia serpentina*). American Journal of Plant Sciences. 2011;3:437-442.
14. Martin KP. Rapid propagation of *Holostemma ada-kodien* Schult., a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. Plant Cell Reports. 2002;21:112-117.
15. Mishra Y, Usmani G, Mandal AK. *In vitro* cloning of *Rauvolfia serpentina* (L.) Benth. Var. CIM-Sheel and evaluation of its field performance. Journal of Biological Research. 2010;13:85.
16. Mittal B, Meenakshi A, Sharma Gothecha VK. Phytochemical and pharmacological activity of *Rauwolfia serpentina* - A review. International Journal of Ayurvedic and Herbal Medicine. 2012;2(3):427-434.
17. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum. 1962;15:473-492.
18. Nemeth G. Induction of rooting. In: Bajaj YPS, ed. Biotechnology in Agriculture and Forestry. Vol. 1: Trees I. Berlin: Springer-Verlag; c1986. p. 49-64.
19. Pandey VP, Kudakasseril J, Cherian E, Patani G. Comparison of two methods for *in vitro* propagation of *Rauwolfia serpentina* from nodal explants. Indian Drugs. 2007;44(7):514.
20. Pant KK, Joshi DS. Rapid multiplication of *Rauwolfia serpentina* Benth. Ex. Kurz. through tissue culture. Scientific World. 2008;6(6):58-62.
21. Panwar GS, Attitalla IH, Guru SK. An efficient *in vitro* clonal propagation and estimation of reserpine content in different plant parts of *Rauwolfia serpentina* L. American-Eurasian Journal of Scientific Research. 2011;6(4):217-222.
22. Patil VM, Jayanthi M. Micropropagation of two species of *Rauwolfia* (Apocynaceae). Current Science. 1997;72(12):961-965.
23. Pospisilova J, Ticha I, Kadlecek P, Haisel D, Plzakova S. Acclimatization of micropropagated plants to ex vitro conditions. *Biologia Plantarum*. 1999;42:481-497.
24. Prakasha D, Ramya G, Nidagundi R. *In vitro* micropropagation of sarpagandha (*Rauvolfia serpentina*). Vitro. 2017;12:1391-1395.
25. Rani A, Kumar M, Kumar S. Effect of growth regulators on micropropagation of *Rauvolfia serpentina* (L.) Benth. Journal of Applied and Natural Science. 2014;6(2):507-511.
26. Rashmi R, Trivedi MP. Rapid *in vitro* regeneration of an endangered medicinal plant sarpagandha (*Rauvolfia serpentina* L.). European Journal of Pharmaceutical and Medical Research. 2016;3(5):276-284.
27. Roja G, Heble MR. Indole alkaloids in clonal propagules of *Rauwolfia serpentina*. Plant Cell, Tissue and Organ Culture. 1996;44(2):111-115.
28. Selvanpandiyan A, Subramani J, Bhatt PN, Mehta AR. A simple method for direct transplantation of cultured plants to the field. Plant Science. 1998;56:81-83.
29. Shekhawat MS, Manokari M. Efficient *in vitro* propagation by ex vitro rooting methods of *Artemisia absinthium* L., an ethnobotanically important plant. Chinese Journal of Biology; c2015 .p. 1-8.
30. Singh P, Singh A, Shukla AK, Singh L, Pande V, Nailwal TK. Somatic embryogenesis and *in vitro* regeneration of an endangered medicinal plant sarpgandha (*Rauwolfia serpentina* L.). Life Science Journal. 2009;6(2):57-62.
31. Sreekumar S, Seeni S, Pushpangadan P. Micropropagation of *Hemidesmus indicus* for cultivation and production of 2-hydroxy 4-methyl benzaldehyde. Plant Cell, Tissue and Organ Culture. 2000;62:211-218.
32. Van der Krieken WM, Breteler H, Visser M HM, Mavridou D. The role of conversion of IBA into IAA on root regeneration in apple: introduction of a test system. Plant Cell Reports. 1993;12:203–206.
33. Vengadesan G, Pijut PM. *In vitro* propagation of northern red oak (*Quercus rubra* L.). *In vitro* Cellular & Developmental Biology - Plant. 2009;45:474–482. DOI:10.1007/s11627-008-9182-6.
34. Wahurwagh A, Deshmukh AG, Padole D, Jadhav PV, Moharil MP. *In vitro* regeneration of medicinal plant sarpagandha (*Rauwolfia serpentina* L.). The Pharma Innovation Journal. 2022;11(6):24-29.
35. Yan H, Liang C, Yang L, Li Y. *In vitro* and ex vitro rooting of *Siratia grosvenorii*, a traditional medicinal plant. Acta Physiologiae Plantarum. 2010;32:115-120.