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Laxmi Mastiholi
Department of Plantation, Spices, Medicinal & Aromatic Crops, UAHS, Shivamogga, Karnataka, India

Raviraja Shetty G
Assistant Professor
Department of Plantation, Spices, Medicinal & Aromatic Crops, UAHS, Shivamogga, Karnataka, India

Souravi K
Division of Plant Genetic Resources, ICAR-IIHR, Bangalore, Karnataka, India

Rajasekharan PE
Principle Scientist
Division of Plant Genetic Resources, ICAR-IIHR, Bangalore, Karnataka, India

Correspondence
Laxmi Mastiholi
Department of Plantation, Spices, Medicinal & Aromatic Crops, UAHS, Shivamogga, Karnataka, India

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In-vitro conservation studies in *Salacia chinensis* L. a threatened medicinal plant

Laxmi Mastiholi, Raviraja Shetty G, Souravi K and Rajasekharan PE

Abstract
Salacia chinensis L. a high valuable anti-diabetic medicinal plant of Hippocrateaceae family, possess various medicinal properties. Due to the lack of proper cultivation practices, excessive and indiscriminate collection of plant for supplement of global demands on herbal medicine, *S. chinensis* L. is severely threatened in nature. Micropropagation system was developed for commercialization and *in-vitro* conservation. Nodal segments were cultured on MS media supplemented with different growth regulators like BAP, NAA, IAA and KIN among these treatments, combination of BAP (2 mg/l) and NAA (0.8 mg/l) shows higher shoot length (3.40±0.12 cm), number of shoots (3.27±0.07) and number of leaves per explant (7.00±0.17) were observed. The same combination of BAP (2 mg/l) and NAA (0.8 mg/l) shows highest survival per cent, high shoot length, number of shoots per explant and number of leaves under *in-vitro* conservation for six month of period. These efforts could help to propagate plants with high medicinal value and thereby, meeting the demands of the growing population by sustainable use and conservation.

Keywords: *Salacia chinensis* L, micropropagation, nodal segment, *in-vitro* conservation

Introduction
Salacia chinensis L. (*Syn*: *Salacia prinoides*) is one of medicinally important perennial, woody climbing shrub belonging to the family Hippocrateaceae which has since been incorporated into the Celastraceae family. It is widely distributed in the tropical and subtropical areas of the world, especially in India, Sri Lanka, China and South East Asian countries such as Thailand, Indonesia and also in a torrid zone area such as Brazil (Li *et al*., 2008) [10]. In India, it is distributed in Karnataka (rare in semi-evergreen forests of Western Ghats), Kerala (coastal forests of Kollam, Western Ghats of Pathanamthitaa and idukki districts), Southern Orissa and in Maharashtra. It occurs in pockets mainly around the Sahyadri–Konkan corridor area of the northern part of Western Ghats (Patwardhan *et al*., 2014) [14]. It is commonly known as 'Saptarangi' and 'Saptachakra' in Ayurvedic medicine, a traditional medicinal system of India (Govindaraj *et al*., 2009; Singh *et al*., 2010) [6, 18].

Biologically active compounds such as salacinol, kotalanol, neokotalanol, neosalacinol, salaprinol, mangiferin, phenolic glycosides and triterpenes have been isolated from the different parts of plants such as roots, root barks, stems, dried parts and water extraction of the whole plant have been extensively used to treat a variety of ailments such as arthritis, rheumatism, leucorrhoea, inflammation, fever, bronchitis and venereal diseases *etc.* (Inman and Reed, 1997; Li *et al.*, 2008; Sikarwar and Patil, 2012) [7, 10, 17]. The extensive role of this species in treating diseases like diabetes, obesity, liver disorder, inflammation, useful as astringent, abortifacient, carminative, emmenagogue, blood tonic, blood purifier, cardio-tonic, amenorrhea and dysmenorrhoea is also been well documented (Govindaraj *et al*., 2009; Matsuda *et al*., 2002; Yoshikawa *et al*., 2001) [6, 13, 20].

Due to the lack of proper cultivation practices, destruction of plant habitats, excessive and indiscriminate collection of medicinal plants for supplement of global demands on herbal medicine, *S. chinensis* L. is severely threatened in nature. In order to conserve and rapidly propagate the rare and endangered medicinal plants, advanced biotechnological tools of culturing plant cells and tissues through micro propagation and *in-vitro* conservation are employed. Since fruit set and germination is a major problem in multiplying the species, there is a need to standardize alternative means of propagation for the production of true to type plants and *in-vitro* conservation.

Materials and Methods

Plant material and cultural conditions

Nodal segments are collected from *S chinensis* raised in ICAR-Indian Institute of Horticulture Research, Field Gene Bank Bangalore. The nodal explants were surface sterilized by placing explants in beakers and covered with muslin cloth and washed for 30 minutes under running tap water to remove all the adhering dust particles and microbes from the surface. The explants were then washed with liquid detergent (Teepol) for another 15 minutes and then washed properly in running tap water to remove detergent. Thereafter, the explants were submerged in 70 per cent (v/v) ethanol for 1-2 minutes; alcohol was decanted by washing the explants with sterile, distilled water and surface sterilized with 0.1 per cent of mercuric chloride ($HgCl_2$) for 5 minutes under the sterile conditions. After that surface disinfected, explants were inoculated on basal MS medium containing different combinations of growth regulators. All the cultures were incubated at temperature of 25 ± 2^0 C under white fluorescent light with 50µ mole m^{-2} s^{-2} light intensity during a photoperiod of 16 hour light and 8 hour dark.

In-vitro conservation

Short term conservation of *in-vitro* raised plants of *S. chinensis* was attempted for six months to slow down its growth by reducing further sub-culturing and providing limited light intensity for growth. In order to accomplish this, equal number of eight week old tissue cultured plants from each treatment (MS medium + Hormonal combinations) were taken and kept under low light intensity (2.97 μm^{-2} s^{-1}) in a chamber having ambient temperature maintained at 10^0 C. Equal numbers of replicates from each treatment were kept under Standard culture conditions (SCC) in order to compare it with those which are kept under Reduced culture condition (RCC). Before transferring the replicates from each treatment for *in-vitro* conservation, proper sub-culturing was done. Observations on growth parameters were recorded at regular intervals.

Result and Discussion

In-vitro shoot multiplication

Nodal bud segments from IIHR- Field Gene Bank plants of *S. chinensis* were used as explant and inoculated to MS media containing different combinations and concentrations of growth regulators *viz.*, BAP, NAA, IAA and KIN. Effect of varying plant growth regulators on growth related characters like shoot length, number of shoots and number of leaves was observed and recorded. Estimation of growth parameters was done after twelve weeks of inoculation. Data were statistically analysed by analysis of variance and significance was calculated. MS medium supplemented with BAP (2 mg/l) and NAA (0.8 mg/l) shown significantly high shoot length (3.40±0.12 cm), number of shoots (3.27±0.07), as well as high number of leaves per explant (7.00±0.17) followed by BAP (1.0 mg/l): NAA (0.5 mg/l). which shows shoot length (3.27±0.09.), number of shoots (3.03±0.09) and number of leaves per explant (6.80±0.10) (Table. 1) This result was found to be consistent to the findings of Chavan *et al*. (2015) [1] and Majid *et al*. (2016) [11] who concluded that multiple shoots can be obtained using basal matured nodal region as explants in *S. chinensis.*

The results indicate that BAP has better potential to promote micropropagation in *S. chinensis* than KIN and supplementation of NAA was more advantageous than IAA. Similar results were reported with *Gymnema sylvestre* and *Ocimum gratissimum* (Gopi *et al*., 2006; Kumar *et al*., 2002) [5, 9]. A combined effect of auxins and BAP on promotion of shoot multiplication is well noticed for medicinal plants (Dangi *et al*., 2014; Martin, 2002) [3, 12]. BAP was most effective for shoot initiation to overcome the apical dominance, releasing lateral buds from dormancy and promotion of the shoot formation is the main reported effects of BAP (George *et al*. 1993) [4]. The prominent effect of BAP on multiple shoot induction has been proved earlier in many medicinal plant including *Talinum triangulare*, *Mentha arvensis* L., *Portulaca oleracea* L. and *Ocimum basilicum* L. (Chishti *et al*., 2006; Safdari *et al*., 2009; Sahoo and Chand, 1998 and Swarna and Ravindhran, 2012) [2, 15, 16, 19]. Rate of cell division might be increased in the axillary and terminal meristematic zone of explant tissues at the presence of BAP. Large number of shoots is produced because of faster pace division of cells in this zone.

Table 1: *In-vitro* shoot multiplication in *Salacia chinensis* L. using different plant growth regulators after 12 weeks of inoculation.

Sl. No.	MS Media + Growth regulators (mg/l)	Shoot length (cm) (Mean ± SE)*	Number of shoots per explants (Mean ± SE)*	Number of leaves per explants (Mean ± SE)*
1	BAP (2.0)	2.63±0.09	2.33±0.03	5.80±0.10
2	KIN (2.0)	2.40±0.06	2.10±0.10	5.43±0.13
3	BAP (0.8)+IAA (0.3)	2.80±0.06	2.53±0.03	6.00±0.17
4	BAP (1.5)+IAA (1.0)	3.13±0.09	2.83±0.12	6.53±0.23
5	BAP (2.0) + NAA (0.8)	3.40±0.12	3.27±0.07	7.00±0.17
6	BAP (1.0)+ NAA (0.5)	3.27±0.09	3.03±0.09	6.80±0.10
7	BAP (1.0) + IAA (0.5)	3.03±0.07	2.70±0.06	6.33±0.20

In-vitro conservation

The objective to go for *in-vitro* conservation by providing, low light intensity (2.97 μm^{-2} s^{-2}) and maintaining a temperature of 10^0 C was to reduce the growth and other growth related traits in order to conserve it for six months and analysing the survival per cent, shoot length, number of shoots and number of leaves after six months of conservation. Krishnan *et al*. (2011) [8] reported the status of medicinal plants of Western Ghats of India and concluded that not only *in-situ* methods but also *ex-situ* methods through biotechnological tools are required to conserve those important medicinal plant species.

Table 2: Comparisons of growth for tissue cultured plantlets kept under Standard Culture Condition (SCC) and Reduced Culture Condition (RCC) after six months.

Sl. No.	MS Media + Growth regulators (mg/l)	Survival (%)		Shoot length (cm) (Mean ± SE)*		Number of shoots per explant (Mean ± SE)*		Number of leaves per explant (Mean ± SE)*	
		SCC	RCC	SCC	RCC	SCC	RCC	SCC	RCC
1	BAP (2.0)	70	50	3.10±0.10	2.63±0.03	2.57±0.03	1.90±0.10	7.33±0.20	5.13±0.30
2	KIN (2.0)	60	40	2.93±0.13	2.50±0.06	2.23±0.13	1.67±0.20	6.97±0.33	4.90±0.10
3	BAP (0.8)+ IAA (0.3)	80	70	3.37±0.07	2.80±0.10	2.80±0.06	2.10±0.10	7.53±0.23	5.43±0.13
4	BAP (1.5)+ IAA (1.0)	85	70	3.77±0.12	3.03±0.12	3.37±0.09	2.43±0.13	8.10±0.10	6.33±0.20
5	BAP (2.0) + NAA (0.8)	100	85	4.10±0.12	3.40±0.06	3.90±0.06	3.00±0.17	8.67±0.20	7.20±0.10
6	BAP (1.0)+ NAA (0.5)	95	80	3.93±0.09	3.27±0.03	3.67±0.07	2.80±0.10	8.43±0.13	6.67±0.20
7	BAP (1.0) + IAA (0.5)	75	60	3.50±0.06	2.93±0.09	3.20±0.12	2.23±0.23	7.80±0.10	5.87±0.30

Fig 1: Standard Culture Condition (SCC) and Reduced Culture Condition (RCC)

Fig 2: Comparisons of growth for tissue cultured plantlets **kept** under Standard Culture Condition (SCC) and Reduced Culture Condition (RCC)

Comparisons of growth for tissue cultured plantlets kept under Standard Culture Conditions (SCC) and Reduced Culture Condition (RCC) after six months.

A comparison in terms of survival per cent, shoot length, number of shoots and number of leaves was made between *in-vitro* conserved plantlets and tissue cultured plantlets regenerated in SCC. It was found that MS medium supplemented with BAP (2.0 mg/l) + NAA (0.80 mg/l) showed highest survival per cent, shoot length, number of shoots and number of leaves in both *in-vitro* conserved plantlets and tissue cultured plantlets regenerated in normal ambient conditions. This showed that, this particular hormonal combination and concentration may be used for short term conservation as well as normal growth of tissue cultured nodal segment.

It was found that, after six month of storage, *in-vitro* raised plants maintained at 10^0 C showed slower growth in comparison to *in-vitro* plants maintained at standard culture condition. Hence, it can be concluded that short term conservation is effective to maintain *in-vitro* cultured plants for prolonged periods without subculture.

Conclusion

Seed germination is a major problem in multiplying the species, there is a need to standardize alternative means of propagation for the production of true to type plants. Tissue culture is a faster and efficient method of propagation to overcome high heterozygosity which in turn can contribute to the *in vitro* conservation effort. An efficient in vitro regeneration system has been developed for medicinally important woody plant, *S. chinensis* using mature nodal explants. This protocol imparts an efficient and successful technique that can be utilized for large-scale propagation, commercialization and ex situ conservation of this important medicinal plant. These efforts could help to propagate plants with high medicinal value and thereby, meeting the demands of the growing population by sustainable use and conservation.

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