

Regular Article

***In vitro* propagation of *Solidago virgaurea* L. through nodal culture**

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A reliable *in vitro* propagation protocol has been established from nodal segments of a highly valuable medicinal plant *Solidago virgaurea* L. The explants were cultured on Murashige and Skoog's medium augmented with different concentrations and combinations of plant growth regulators for shoot bud initiation and multiplications. Highest frequency of shoot proliferation (91.8 ± 0.64) and maximum number (12.1 ± 0.20) per node was observed in Murashige and Skoog's medium augmented with 4.5 mg/l of Kinetin in combination with 0.5 mg/l of Indole -3- Acetic acid. Highest frequency (91.7 ± 1.43) of rooting and maximum number (3.8 ± 0.28) of rootlet per shoot let was achieved on Murashige and Skoog's medium fortified with 4.5 mg/l of Indole -3- Acetic acid in combination with 0.5 mg/l of Benzyl -6- amino purine. Regenerated plants were successfully transferred to field (78%).

Keywords: *in vitro*; Clonal; Nodal segments; Medicinal plants

Abbreviations: MS - Murashige and Skoog's medium; PGRs - Plant Growth Regulators; BAP - Benzyl Amino Purine; IAA - Indole -3-Acetic Acid; IBA - Indole -3- Butyric Acid; NAA - α Naphthalene Acetic Acid; Kin - Kinetin

Plants are the main source of medicines and play a key role in world health and global economy (Constable 1990; Srivastava *et al.*, 1995). In the past few decades there has been a resurgence of interest in the study and use of medicinal plants in health care and in recognition of the importance of medicinal plants to the health system (Lewington 1993; Mendelsohn and Balick 1994; Hoareau and DaSilva 1999). This awakening has led to a sudden rise in demand for herbal medicines, followed by a belated growth in international awareness about the dwindling supply of the world's medicinal plants (Bodeker 2002). Most of the pharmaceutical industry is highly dependent

on wild populations for the supply of raw materials for extraction of medicinally important compounds. The genetic diversity of medicinal plants in the world is getting endangered at an alarming rate because of ruinous harvesting practices and over-harvesting for production of medicines, with little or no regard to the future (Nawalde and Tsay 2004). Also, extensive destruction of the plant-rich habitat as a result of forest degradation, agricultural encroachment, urbanization, etc. are other factors. Hence there is a strong need for proactive understanding in the conservation, cultivation, and sustainable usage of important medicinal plant species for future

use (Nawalde and Tsay 2004). During the last few years, *in vitro* culture techniques have been developed into a successful and rapid mean of asexually propagating a number of plant species. Clonally propagating by tissue culture is highly desirable to regenerate sufficient populations of plants with similar characteristics, decreasing or eliminate the possibility of anomaly what occurring with others methods (Bajaj *et al.*, 1988). Also, plant tissue culture is useful for conservation and rapid propagation of rare and endangered medicinal plants. Regeneration through micropropagation has been obtained in many medicinal species (Johnson *et al.*, 2002; Johnson and Manickam 2003; Johnson *et al.*, 2004; Benniamin *et al.*, 2004; Johnson *et al.*, 2005; Baskaran and Jayabalan 2005; Johnson 2006; Johnson *et al.*, 2007; Johnson 2007; Rout *et al.*, 2007; Johnson *et al.*, 2009; Vijayakumar *et al.*, 2010; Chandra Prabha Rama Subbu 2010; Johnson *et al.*, 2010; Shukla *et al.*, 2011; Wesely *et al.*, 2010; Pandeya *et al.*, 2010; Okere and Adegeye 2011; Wesely *et al.*, 2011). There are no previous reports on micropropagation of any species of *Solidago virgaurea*. So, the objective of this investigation was to develop efficient systems for the *in vitro* propagation of this medicinal species *Solidago virgaurea* L. by apical and nodal segment proliferation.

Plants of *Solidago* (fam. Asteraceae) genus contain terpenoids, saponins, phenolic acids, phenolic glycosides and high amounts of flavonoids, mainly quercetin, kaempferol, and rutin (Lorenzi and Matos, 2002; Reznicek *et al.*, 1991). *Solidago* species have been used in folk medicine for the treatment of various inflammatory and infectious processes. Studies using *in vitro* and *in vivo* models have demonstrated that plants of this genus have important antimicrobial, analgesic, antineoplastic and antioxidant effects. It is recommended as a diuretic, analgesic and anti-inflammatory to treat

burns and rheumatic disease, among other conditions. From the chemical point of view, flavonoids and their derived compounds have been isolated from the *Solidago* sps. Besides their anti-inflammatory activity effect, flavonoids have been demonstrated to have important antimicrobial and antiplatelet aggregation properties *Solidago virgaurea* L. is an herbaceous perennial plant of the family Asteraceae. It has been traditionally used to treat urinary tract, nephrolithiasis and prostate. The plant contains triterpene saponins, flavonoids (derivatives of quercetin, kaempferol and apigenin), polyphenolic acids (ferulic and chlorogenic), tannins, essential oil and polysaccharides. In addition, plant possesses antimicrobial, antimycotic, anti-oxidant, anti-inflammatory, analgetic, anticancerogenic, sedative, and hypotensive activities (Ivancheva and Stancheva, 2000; Thiem and Goslinska, 2002; Demir *et al.*, 2009). The crude ethanolic and methanolic extracts of *S. virgaurea* showed a moderate bactericidal activity (Gross *et al.*, 2002).

The Indian system of medicine is predominantly dependent upon the use of plant based raw materials in most of their preparations and formulations, thereby, widening the gap between demand and supply and thus putting further pressure on the species. In view of the problems of conventional propagation and high demand of planting material the large scale multiplication of this species can only be met efficiently and economically in a short span of time by *in vitro* propagation. Therefore, an efficient *in vitro* propagation system for producing this plant *S. virgaurea* is required to further clarify its potential medicinal values and germplasm conservation. Thus the present study has been intended to develop a trustworthy and reproducible protocol of this important medicinal plant *S. virgaurea* using nodal segments as explants which could be used for mass multiplication

of this plant species to meet the increasing requirement of the pharmaceutical industry as well as for the conservation of *S. virgaurea*.

Materials and Methods

Plants of *Solidago virgaurea* L. (Asteraceae) collected from the native habitats at Tirunelveli, Tamil Nadu, India and grown in the Botanical garden of Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India. Young shoots were harvested and washed with running tap water and surface sterilized in 0.05 and 0.1% mercuric chloride for 2, 3 and 5 min. After rinsing 3-4 times with sterile distilled water, nodal segments were separated and cut into smaller segments (0.5 to 1.0 cm) used as the explants. The explants were placed vertically (nodal segments) on solid basal Murashige and Skoog (1962) medium supplemented with 3% sucrose, 0.7% (w/v) agar (Hi-Media, Mumbai) and different concentration (0.5-2.0 mg/l) and combination of BAP, Kin and IAA for *in vitro* shootlets and rootlets regeneration. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 min. The cultures were incubated at 25 ± 2°C under cool fluorescent light (2250 lux 12 hr/d photoperiod). Each and every experiment was performed with 20 replicates and repeated twice. For hardening, the *in vitro* raised plantlets were removed from culture, washed thoroughly with tap water planted in small polycups filled with sterile garden soil (3:1), covered by unperforated polybags, and hardened for 4 weeks in a mist chamber before transfer to field.

Results and Discussion

The surface sterilization of *S. virgaurea* was carried with different concentration of mercuric chloride such as 0.05%, 0.1% and 0.15% for different time

duration. Among them, 0.1% mercuric chloride for 3 min showed low percentage contamination and highest (92.3%) percentage of microbes / contaminants free explants. The explants treated with 0.05% and 0.15% of mercuric chloride for 3 min showed 55- 60% of microbes free explants. The explants treated with 0.1% for above 3 min and 0.15% for 2½ min and above obtained hundred percentages of microbes free explants with high percentage of explants mortality, high concentration of mercuric chlorides leads the death of the explants (lethal effect). Singh *et al.* (2009) observed that 5.5 ± 2.12 percentage of contamination when the explants were treated with 0.1 % (w/v) mercuric chloride for 5 min and 40 % (v/v) Sodium hypochlorite for 20 min. Wesely *et al.*, (2011) observed that the explants treated with 0.1% mercuric chloride for 3½ min showed 4% microbial contamination. In the present study we observed that 3 min sterilization with 0.1% HgCl₂ showed 7.7% of contamination with less percentage of explants mortality rate. The medium (MS) augmented with different concentrations and combinations of plant growth regulators (BAP (0.5- 5 mg/l), Kin (0.5- 5 mg/l) and IAA (0.5- 5.0 mg/l)) were used for multiple shoots emergence from the shoot tip and nodal segments. The explants (nodal segments) of *S. virgaurea* started growing in MS medium supplemented with BAP and Kin in combination with IAA within a week. Highest percentage (91.8 ± 0.64%) and maximum number (12.1 ± 0.20) of shoot induction from nodal segment was observed on MS medium supplemented with 4.5 mg/l of Kin in combination with 0.5 mg/l of IAA (Fig. 1. A & B) (Table 1). The MS medium augmented with cytokinin alone or in combination with auxin (Kin + IAA) induced maximum number of multiple shoots with maximum percentage. The nodal explants cultured on MS medium augmented with

cytokinin (BAP) alone induced multiple shoot formation was observed in *Baliospermum montanum* (Johnson and Manickam 2003), *Adenia hondala* (Johnson et al., 2004), *Passiflora mollissima* (Johnson et al., 2007), *Stevia rebaudiana* (Janarthanam et al., 2009), *Vitex negundo* (Islam et al., 2009), *Marsdenia brunoniana* (Ugriah et al., 2010) and *Alternanthera sessilis* (Wesely et al., 2011). The *in vitro*-raised shootlets were transferred to half-strength MS medium with different concentrations of BAP, Kin, IBA, IAA and NAA for rooting (Table 1). Highest percentage ($91.7 \pm 1.43\%$) and maximum

number (3.8 ± 0.28) rootlets were observed on MS medium augmented with 4.5 mg/l of IAA in combination with 0.5 mg/l of BAP (Fig. 1. C). In the present study, we observed the highest percentage and maximum number of rootlets per shootlets on half strength medium supplemented with 3 mg/l IBA. The cytokinin in combination with auxin promoted shoot formation in various plant species as observed by Yasmeen and Rao (2005), Guo et al., (2007) Jawahar et al., (2008), Nisha et al., (2009), Ioan Băcilă et al., (2010) and Yadav and Singh (2010).

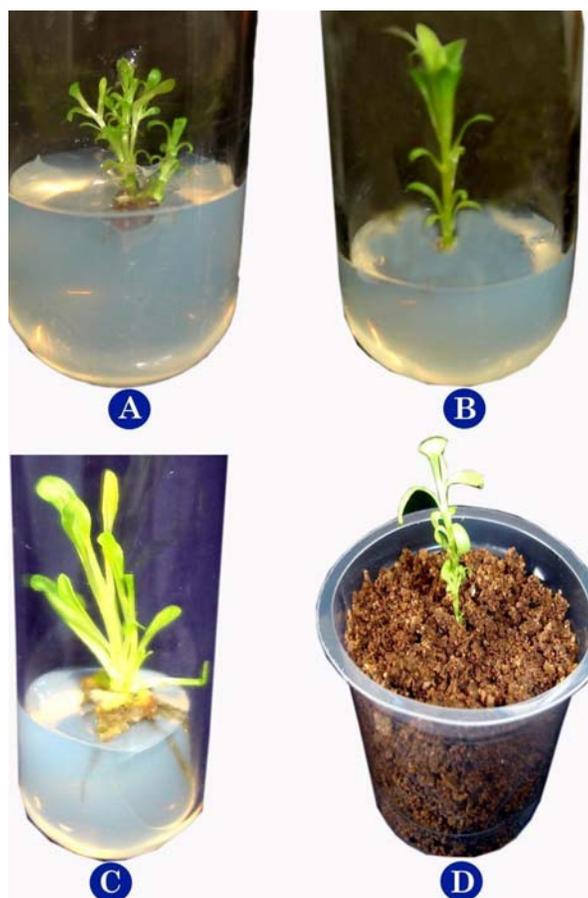


Fig.1. *In vitro* propagation of *Solidago virgaurea* L

A. Multiple Shootlets formation from the nodal segment of *S. virgaurea* cultured on MS+Kin 4.5 mg/l + IAA 0.5 mg/l; B. Shoot Elongation of *S. virgaurea* cultured on MS+Kin 4.5 mg/l + IAA 0.5 mg/l; C. *In vitro* rootlets formation from the *in vitro* derived shootlet of *S. virgaurea* cultured on MS+BAP 0.5 mg/l + IAA 4.5 mg/l; D. Hardened Plantlet of *S. virgaurea*

Table 1: Influence of Plant Growth Regulators on multiple shootlets and rootlets formation of *Solidago virgaurea* L.

Plant Growth Regulators Concentration in mg/l			Mean % of Shootlets formation \pm S.E.	Mean No. of shootlets /nodal segments \pm S.E.	Mean % of Rootlets formation \pm S.E.	Multiple Rootlets /Shootlet \pm S.E.	Degree of Callus formation
IAA	Kin	BAP					
0.5	4.5	0.0	91.8 \pm 0.64	12.1 \pm 0.20	-	-	-
1.0	4.0	0.0	83.8 \pm 0.53	9.4 \pm 0.36	-	-	-
1.5	3.5	0.0	72.4 \pm 0.46	2.3 \pm 0.24	-	-	+
2.0	3.0	0.0	63.7 \pm 0.39	1.4 \pm 0.18	-	-	++
2.5	2.5	0.0	45.6 \pm 0.54	1.1 \pm 0.31	-	-	++
4.5	0.0	0.5	48.4 \pm 0.29	1.2 \pm 0.23	91.7 \pm 1.43	3.8 \pm 0.28	-
4.0	0.0	1.0	54.4 \pm 0.62	1.5 \pm 0.36	83.8 \pm 1.27	3.6 \pm 0.33	-
3.5	0.0	1.5	61.3 \pm 0.43	1.6 \pm 0.53	78.3 \pm 0.96	2.8 \pm 0.44	++
3.0	0.0	2.0	63.8 \pm 0.46	1.7 \pm 0.42	65.8 \pm 0.48	2.1 \pm 0.52	++
2.5	0.0	2.5	65.3 \pm 0.56	2.3 \pm 0.56	56.9 \pm 1.12	1.8 \pm 0.63	++

+ - sign indicates average callus induction; ++ - sign indicates high amount of callus induction

In the present study the auxin in combination with cytokinin (BAP) accelerated the rootlets formation. Similar to the present study observation Marta *et al.*, 2009; Taware *et al.*, 2010; Kalidass *et al.*, 2010 also were observed the BAP in combination with IAA promoted rootlets formation in different plants. After 30 days, *in vitro*-raised plantlets were hardened in polycups containing a mixture of sterile garden soil: sand (3:1), covered with polypropylene bags and irrigated with 10 X diluted MS liquid medium. The plants were kept in a culture room for 15 days. 63% of plants were successfully established in polycups (Fig. 1. D). After 15 days the polycups hardened plants were transferred to pots and kept in green house. Seventy four percent of plants were well established in the green house condition. After one month, the plants were transferred to the field. About 67% of plants were established in the field. The present study has established reliable and repeatable protocol for large scale multiplication of *S.*

virgaurea through nodal segments. This protocol could be used as a tool for the large scale multiplication.

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