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Short Communication

An efficient protocol devised for rapid callus induction from leaf explants of *Stephania wightii* (Arn.) Dunn. - an endemic medicinal plant

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Callus culture of *Stephania wightii* (Arn.) Dunn. was established from leaf explants. Different growth regulators greatly influenced the growth of callus cultures. Leaf derived callus grown on Murashige and Skooge (MS) medium fortified with different concentrations (0.1-2.5 mg/L) of Thiodiazuran (TDZ), 6-Benzyladenine (BA), 2, 4- Dichlorophenoxyacetic Acid (2, 4-D) and α-Naphthalene Acetic Acid (NAA). Maximum callus growth was achieved in medium supplemented with TDZ + NAA (1.5+0.02 mg/L) was found to be more suitable than other growth regulators used.

Keywords: *Stephania wightii,* callus, leaf explant and growth regulators.

The genus *Stephania* belongs to the family Menispermaceae, a large family which consist of about 65 genera and 350 species, distributed in warmer parts of the world. *Stephania wightii* (Arn.) Dunn. of this family Menispermaceae, is a slender climber with peltate and membranous leaves. It has been used in folk medicine for the treatment of various ailments such as asthma, tuberculosis, dysentery, hyperglycemia, malaria, cancer and fever (Krithikar and Basu, 2004). The reported data showed that alkaloids are the main common phytochemicals of the genus *Stephania*. More than 200 alkaloids have been isolated from this genus together with flavonoids, lignans, steroids, terpenoids and coumarins. About ten thousand plant alkaloids have been reported in the genus *Stephania* and many of these pharmacologically active alkaloids are mostly involved in plant defense against pathogens, insects, and herbivores. Their potent toxicity makes alkaloids "privileged structures for drug development (Leonard et al. 2009). This plant becomes endemic due to the loss of habitat, poor perpetuation, poor germination rate, limited distribution, over harvesting and pollution. Conservation of this endemic medicinal plant is therefore important to ensure sustainable human development. In the present study, *in vitro* technique was chosen to develop and conserve this medicinally important plant for future.

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Material and methods

Plant material and explants source:

The tuber of *Stephania wightii* was collected from Wyanad, Kerala and brought to Coimbatore and grown under green house conditions was used for this study. The leaf explants were thoroughly washed under running tap water for 25-30 min and then rinsed in a solution containing the surfactant Tween-20 (2 drops in 100 ml solution). Subsequently, they were surface sterilized with 0.1% (w/v) $HgCl_2$ solution for 0.5-1 min, followed by 3 to 5 rinses with sterile distilled water in a clean air cabinet. The surface-sterilized explants were aseptically cut into 1-2 cm segments and carefully inoculated onto MS culture media (Murashige and Skoog, 1962). In this study, analytical grade chemicals procured from Hi Media Pvt. Ltd. Mumbai, India were used.

Culture media and culture conditions:

The culture media consisted of MS salts augmented with 3% (w/v) sucrose and gelled with 0.8% (w/v) agar (Hi-Media, India). The MS medium is supplemented with combination of various auxins and cytokinins. All plant growth regulators were added to the medium before autoclaving. The pH of the medium was adjusted to 5.6 to 5.8, followed by autoclaving at 121°C at 15 psi (1.06 kg/cm2) pressure for 15 to 20 minutes. The cultures were incubated at 25 ± 2 °C and 60 µmol m^{-2} s^{-2} light intensity under 12 h photoperiod with cool-white fluorescent tubes (Philips, India) and 55% relative humidity.

Statistical analysis:

A minimum of ten culture tubes were developed for all combinations previously explained. Analysis of variance (ANOVA) and mean separations were carried out using Duncan's multiple range test, followed by Tukey's multiple comparison tests to assess the statistical significance. $P \leq 0.05$ was considered to be statistically significant, using statistical software SPSS ver. 14 (SPSS Inc., Chicago, USA).

Results and discussion

MS medium supplemented with different concentrations of growth regulators TDZ, BA, 2,4-D and NAA used for callus induction. Results indicated that all the growth regulators alone not able to induce a callus from *S. wightii* leaf explants. While the combinations of growth regulators show maximum callus production. The young moderate sized leaf explants were well responded for rapid callogenesis after incubation period of about 4 weeks. A Pale green colour and healthy compact calli were observed on First week of inoculation at the trimmed regions on MS media supplemented with 1.5mg/l TDZ concentrations. For successful callus initiation, substantiation of the basal media (supplemented with 3% sucrose) with 1.5+0.02 mg/l TDZ + NAA was found to be more suitable than other growth regulators used. Whereas, BA at 1.5 mg/l concentration and 2, 4-D at 2.0 mg/l concentration showed white and fragile callus. Lowest callus fresh weight was obtained when MS medium was supplemented with 2, 4-D. However, highest callus fresh weight was obtained in the medium containing 1.5 mg/l TDZ + 0.02 mg/l NAA (Table 1 and Fig 1).

One of the most important factors governing callogenesis is the composition of the culture medium. MS medium showed the highest percentage and dry weight of the callus in *S. wightii*. Khatun et al. (2003) have reported that MS was the most suitable media for callus induction. *S. tetrandra* grown on different basal salt media obtained maximum callogenesis using one fold MS media (Yang and Lu, 2006). Addition of peptone into the culture medium has strongly improved the growth of tobacco callus (Parc et al. 2007). In this study, the addition of peptone (2 g/L) promoted callus growth. Because peptides could be more

efficient source of nitrogen for sustaining *in vitro* callus proliferation.

The texture of callus varied according to the nature of cytokinin and also on auxin: cytokinin ratio (Martin, 2002). Morphological and phytochemical differences in callus are attributing to culture conditions, composition of the medium (Torrey, 1966), orientation of the explant (Mathysse and Torrey, 1967), and growth and morphogenesis ability of plants itself. Type and concentration of hormone present in medium directly affect the nature, colour and biochemical composition of callus (Skoog and Miller, 1957). Variations in the callus forming ability of different explant types, has been reported in many plants (Ishii et al., 2004).

Table 1. Effect of different concentrations of plant growth regulators on callus induction from leaf of *Stephania wightii.*

Plant growth regulators	Concentration of plant growth regulators (mg/L)	Leaf explant		
		Intensity of callus formation	Mean callus weight ± SD	Nature of callus
Control	--	--	--	--
TDZ	0.1	+	0.42±0.06	White green, friable
	0.5	+	0.47±0.04	White green, friable
	1.5	+	0.61±0.50	White green, friable
	2.0	++	0.84±0.42	Light green, compact
	2.5	++	0.74±0.31	Light green, compact
BA	0.1	--	0	No callus formed
	0.5	--	0	No callus formed
	1.5	+	0.37±0.07	White green, friable
	2.0	+	0.42±0.23	White green, friable
	2.5	++	0.79±0.15	Light green, compact
2,4-D	0.1	--	0	No callus formed
	0.5	--	0	No callus formed
	1.5	--	0	No callus formed
	2.0	+	0.21±0.09	White green, friable
	2.5	+	0.26±0.06	White green, friable
TDZ+ NAA	0.1+0.02	+	0.67±0.43	White green, friable
	0.5+0.02	+	0.73±0.50	White green, friable
	1.5+0.02	+++	1.53±0.18	Light green, compact
	2.0+0.02	++	0.87±0.42	White green, friable
	2.5+0.02	+	0.61±0.13	White green, friable
BA+ NAA	0.1+0.02	--	0	No callus formed
	0.5+0.02	+	0.24±0.27	White green, friable
	1.5+0.02	+	0.46±0.05	White green, friable
	2.0+0.02	++	1.15±0.19	Light green, compact
	2.5+0.02	++	0.72±0.24	Light green, compact
2,4-D+ NAA	0.1+0.02	--	0	No callus formed
	0.5+0.02	--	0	No callus formed
	1.5+0.02	+	0.16±0.46	White green, friable
	2.0+0.02	+	0.27±0.50	White green, friable
	2.5+0.02	+	0.34±0.18	White green, friable

Intensity of callus: (+) low; (++) moderate; (+++) high.

Callus from leaf segments showed initiation of vigorous, proliferating, soft and green colored tissue (Chand and Roy, 1980). TDZ, a synthetic phenylurea, is considered to be one of the most active cytokinins for callus and shoot induction in plant tissue culture

(Murthy et al. 1998). TDZ-induced callus induction from different explants of many recalcitrant species as well as from medicinal plants has been reported (Mithila et al. 2003). Several reports suggest that TDZ results in callus induction better than other cytokinins (Thomas, 2003).

Aditi Singh et al. (2009) have explained callus formation in *Tinospora cordifolia* occurred from nodal, inter-nodal and leaf explants when planted on the MS medium containing the combination of BAP and NAA. In this callusing was seen only on leaf explants. Khan et al. (2008) study showed a combination of 2,4-D, BAP and Kin (2.0, 2.0, 1.0 µM) in MS medium enhances the callus production in *Coscinium fenestratum.* Our results are similar to Kuo et al. (2011) investigations, wherean efficient *in vitro* callus induction system in *Stephania tetrandra* was established on MS medium supplemented with 3% sucrose and different concentrations of plant growth regulators i.e. auxins (2,4-D, IAA and NAA) and cytokinins (BA, kinetin, TDZ and zeatin) in the dark. MS medium supplemented with 1.0 mg/L BA and 0.5 mg/L TDZ supported callus growth and its proliferation. A maximum amount of dry biomass (7.8 fold) was produced 45 days after culture. Similarly in *Dioscoreophyllum cumminsii* (Menispermaceae), the stem explants developed brown friable callus in different basal media, supplemented with 2 mg/l of 2,4-D. Callus induction was found to be best in MS media solidified with 10 g/l agar and supplemented with 1-5 mg/l NAA. Further, addition of kinetin (1-5 mg/l) has resulted in more active callus formation. The colour of *D. cumminsii* calluses ranged from brown to green, greenish yellow and yellow (Oselebe and Ene-Obong, 2007).

Conclusion

Vegetative plant parts especially leaves are desirable explants for *in vitro* improvement because of regeneration from these explants would preserve the genetic homozygosity of the parent genotype. Callus culture system offer many advantages as a model system for several biological investigations. Here, in the present investigation an efficient protocol has been devised for *in vitro* callus induction of an endemic medicinal plant, *Stephania wightii* from young leaf explants.

Habit of *Stephania wightii*

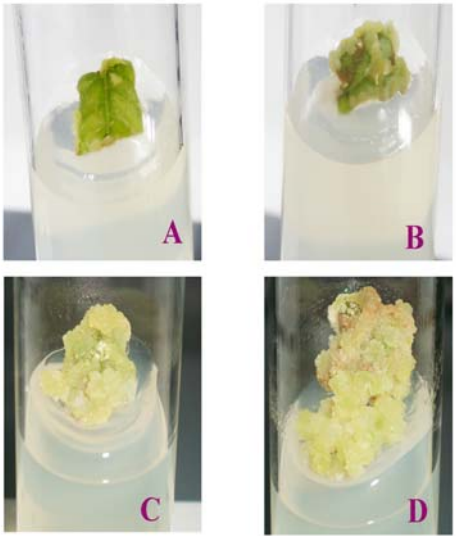

Fig-1 Induction and proliferation of callus from leaf of *Stephania wightii.*

A- Leaf explant on MS medium after 7 days of inoculation.

B- Callus after 4 weeks (MS medium supplemented with TDZ (2.0 mg/L)).

C- Callus after 6 weeks (MS medium supplemented with TDZ with NAA (2.0 + 0.02 mg/L)).

D- Callus after 8 weeks (MS medium supplemented with TDZ with NAA (2.0 +0.02 mg/L)).

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