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In Vitro Shoot Regeneration of *Corchorus acutangulus* Lam. (Tiliaceae).

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ABSTRACT

The herb, *Corchorus acutangulus* is an important medicinal plant belongs to the family Tiliaceae. The present study describes the efficient *in vitro* regeneration protocol standardized for this species through leaf explant. The leaf explants were cultured on Murashige & Skoog (MS) medium supplemented with 2,4-D (1.5 mgl^{-1}) and NAA (0.2 mgl^{-1}) for efficient callus induction (84.43%). Multiple shoots (83%) were developed in the MS medium fortified with IBA (2.0 mgl^{-1}) and TDZ (0.2 mgl^{-1}). Better rooting response (74%) was observed on half strength MS medium containing IBA (2.0 mgl^{-1}) and Kn (0.2 mgl^{-1}). Regenerated plantlets were successfully acclimatized and hardened off inside the culture room and then transferred to green house with 81% survival rate.

INTRODUCTION

Micropropagation offers a great potential for conservation and large scale multiplication of useful medicinal plant species and subsequent exploitation. In addition, *in vitro* culture techniques are required to develop genetic transformation and to establish cell suspension for the production of secondary metabolites. In this respect, vegetative propagation by employing tissue culture technique is worth special attention as a possible alternative to overcome the limited success of more conventional techniques[1,2]. The species, *Corchorus acutangulus* is distributed in the microclimatic areas of river banks where sandy soil is available and also in shade conditions undisturbed areas[3]. Herein it is described the optimization of culture conditions and plant growth regulators required for callus induction, shoot regeneration and rooting of plantlets from immature leaflet of *Corchorus acutangulus*.

METERIALS AND METHODS

Leaf segments from young and healthy branches of *C. acutangulus* were used as explants. They were collected from pot cultured individuals maintained in a mist chamber. For surface sterilization, the collected immature leaves were washed with tap water twice and then treated with 5 % tween-20 solutions for 5 min followed by rinsing in tap water. To eliminate fungal contamination, explants were further treated with 5 % antibiotics (Ampicillin and Rifampicin) for 30 min followed by 3 rinses in sterile double distilled water. Further, surface sterilization was carried out by dipping the explants in 0.1% $HgCl_2$ for 3 min followed by 3-4 rinses in sterile double distilled water.

Media and culture condition

Murashige and Skoog[4] (MS) medium containing 3 % sucrose solidified with 1 % agar (tissue culture grade, Himedia, India) was used. The pH of the medium was adjusted to 5.6-5.8 prior to the addition of agar before autoclaving at 121^{0}C for 15 min. All the culture bottles were kept in culture chamber at 25± 2^{0}C under 16/8 hr (light/dark) photoperiod with a light intensity of 2000 lux supplied by cool white fluorescent tubes and with 60-65% relative humidity.

Callus induction medium

The explants were transferred to culture bottles containing 25 mL of MS medium supplemented with different concentrations and combinations of 2,4-D and NAA for callus induction.

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Shoot induction medium

MS medium containing different concentrations and combinations of IBA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and TDZ at 0.2 mg/l was used for shooting attributes.

Rooting of elongated shoots and acclimatization

After proper shoot induction, the plantlets were carefully removed from the medium and washed with sterilized double distilled water properly so as to avoid any trace of medium on roots. *In vitro* regenerated shoots (5-6 cm long) were excised and transferred onto the rooting media containing half strength MS medium supplemented with IBA and Kn for rooting. After proper root formation, these rooted plantlets were transferred to hardening medium composed by garden soil, sand and vermicompost in different proportion and maintained in greenhouse condition to know the survivability rate.

Statistical analysis

All the experiments were done at least twice using triplicate. The data was statistically processed and means were compared using Duncan's Multiple Range Test ($P<0.05$).

RESULTS AND DISCUSSION

Table 1: Effect of growth regulators on callus induction from leaf explants of the species, *Corchorus acutangulus.*

Growth regulators (mg/L)				Days required for callus formation after	Callus formation (%)
BAP	2,4-D	TDZ	NAA	Leaf explant	Leaf explant
0.0	0.5	0.0	0.2	23	59.31^{g} ± 0.82
0.0	1.0	0.0	0.2	23	68.22^{h} ± 1.63
0.0	1.5	0.0	0.2	30	84.43^{j} ± 0.82
0.0	2.0	0.0	0.2	19	79.44^{l} ± 1.63
0.0	2.5	0.0	0.2	26	82.00^{i} ± 1.63
0.0	3.0	0.0	0.2	28	80.18^{i} ± 0.82
0.0	0.3	0.0	0.0	18	15.56^{a} ± 1.63
0.0	0.6	0.0	0.0	19	23.76^{b} ± 0.82
0.0	0.9	0.0	0.0	21	35.97^{c} ± 1.63
0.0	1.2	0.0	0.0	17	51.46^{f} ± 1.63
0.0	1.5	0.0	0.0	23	69.00^{h} ± 0.82
0.5	0.0	0.4	0.0	18	25.21^{b} ± 1.63
1.0	0.0	0.4	0.0	20	41.38^{d} ± 1.63
1.5	0.0	0.4	0.0	22	58.45^{g} ± 1.63
2.0	0.0	0.4	0.0	24	69.64^{h} ± 0.82
2.5	0.0	0.4	0.0	28	78.32^{i} ± 1.63
3.0	0.0	0.4	0.0	27	77.17^{i} ± 1.63
0.0	0.3	0.0	0.2	16	15.89^{a} ± 0.82
0.0	0.6	0.0	0.4	17	23.43^{b} ± 0.82
0.0	0.9	0.0	0.6	18	41.00^{d} ± 0.82
0.0	1.2	0.0	0.8	20	46.00^{e} ± 1.63
0.0	1.5	0.0	1.0	19	47.00^{e} ± 1.63

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT.

Optimization of micropropagation protocol depends upon various factors *viz*., media, explants, growth factors and cultural conditions. Hence in the present study various stages of micropropagation were standardized by screening different factors. The effect of different combinations and concentrations of growth regulators on callus initiation from leaf explants of C. *acutangulus* is shown in Table 1. An effective callus formation (84%) by leaf explants was observed on the MS medium fortified with the growth regulator, 2,4-D (1.5 mgl^{-1}) and NAA (0.2 mgl^{-1}). The results suggested that the auxin, 2,4-D alone, or in combination with NAA produced efficient callus after 30 days of culture with three subcultures. Ganesan and Paulsamy [5] reported that for the species, *Lobelia nicotianaefolia* callus formation was greater in the MS medium containing higher content of 2, 4- D with lower proportion of

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NAA. Various explants responses for callus formation could be due to the various factors viz., nature of explants, growth factors and degree of totipotency. Previous reports also described similar pattern of high degree of calli induction by higher content of 2,-D in MS medium[6,7]. The more pronounced response of leaf explant may be due to the endogenous level of growth regulators as observed in the plant species, *Bupleurum fruticosum*[8], *Bupleurum kaoi* [9], *Anaphalis eliptica*[10].

Table 2: Effect of different concentrations of growth regulators on shoot initiation, shoot number and shoot length after the subculturing of leaf derived callus of the species, *Corchorus acutangulus.*

Growth regulators (mg/L)					Culture response (%)	No. of shoots /callus	Shoot length (cm)
TDZ	IBA	Kn	BAP	GA_3			
0.5	0.0	0.0	0.3	0.0	$29.42^{a} \pm 0.82$	$2.17^{abc} \pm 0.82$	$0.9^{a} \pm 0.82$
1.0	0.0	0.0	0.3	0.0	$52.67^{fg} \pm 1.63$	$3.52^{ab} \pm 1.63$	$2.8^{abc} \pm 0.82$
1.5	0.0	0.0	0.3	0.0	$59.00^{h} \pm 0.82$	$5.23^{bcd} \pm 0.82$	$3.0^{abc} \pm 1.63$
2.0	0.0	0.0	0.3	0.0	$45.78^{c} \pm 1.63$	$7.42^{def} \pm 1.63$	$3.5^{abc} \pm 0.82$
2.5	0.0	0.0	0.3	0.0	$48.24^{de} \pm 0.82$	$6.18^{cde} \pm 1.63$	$2.6^{abc} \pm 0.82$
3.0	0.0	0.0	0.3	0.0	$47.27^{cd} \pm 1.63$	$5.00^{bcd} \pm 0.82$	$3.2^{abc} \pm 1.63$
0.5	0.2	0.0	0.0	0.0	$58.48^{h} \pm 1.63$	$6.65^{cde} \pm 0.82$	$3.8^{abc} \pm 1.63$
1.0	0.2	0.0	0.0	0.0	$63.00^{i} \pm 0.82$	$8.49^{efg} \pm 1.63$	$4.2^{abc} \pm 0.82$
1.5	0.2	0.0	0.0	0.0	$78.43^{j} \pm 1.63$	$7.38^{def} \pm 2.45$	$4.9^{bc} \pm 1.63$
2.0	0.2	0.0	0.0	0.0	$83.01^{k} \pm 0.82$	$13.56^{i} \pm 1.63$	$6.3^{c} \pm 0.82$
2.5	0.2	0.0	0.0	0.0	$63.67^{i} \pm 1.63$	$11.47^{hi} \pm 1.63$	$5.3^{bc} \pm 1.63$
3.0	0.2	0.0	0.0	0.0	$58.46^{h} \pm 1.63$	$10.98^{ghi} \pm 0.82$	$4.7^{abc} \pm 0.82$
0.5	0.0	0.2	0.0	0.0	$53.38^{g} \pm 0.82$	$9.66^{fgh} \pm 0.82$	$4.4^{abc} \pm 1.63$
1.0	0.0	0.2	0.0	0.0	$48.00^{de} \pm 0.82$	$7.22^{def} \pm 1.63$	$3.8^{abc} \pm 0.82$
1.5	0.0	0.2	0.0	0.0	$58.67^{h} \pm 1.63$	$4.16^{abc} \pm 1.63$	$2.7^{abc} \pm 0.82$
2.0	0.0	0.2	0.0	0.0	$62.89^{i} \pm 0.82$	$3.18^{ab} \pm 0.82$	$1.8^{a} \pm 0.82$
2.5	0.0	0.2	0.0	0.0	$68.55^{j} \pm 0.82$	$2.67^{a} \pm 0.82$	$3.0^{abc} \pm 1.63$
3.0	0.0	0.2	0.0	0.0	$59.26^{h} \pm 1.63$	$5.47^{bcd} \pm 0.82$	$3.4^{abc} \pm 0.82$
0.5	0.0	0.0	0.0	0.2	$63.12^{i} \pm 0.82$	$6.65^{cde} \pm 1.63$	$3.6^{abc} \pm 1.63$
1.0	0.0	0.0	0.0	0.2	$80.66^{j} \pm 0.82$	$4.86^{abc} \pm 1.63$	$4.2^{abc} \pm 1.63$
1.5	0.0	0.0	0.0	0.2	$54.37^{g} \pm 0.82$	$2.46^{a} \pm 0.82$	$4.0^{abc} \pm 0.82$
2.0	0.0	0.0	0.0	0.2	$50.47^{ef} \pm 1.63$	$3.14^{ab} \pm 0.82$	$3.8^{abc} \pm 0.82$
2.5	0.0	0.0	0.0	0.2	$46.76^{cd} \pm 1.63$	$5.56^{bcd} \pm 1.63$	$3.0^{abc} \pm 1.63$
3.0	0.0	0.0	0.0	0.2	$42.00^{b} \pm 0.82$	$7.98^{def} \pm 0.82$	$2.9^{abc} \pm 0.82$

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT.

The combination of the cytokinin, of TDZ (2.0 mgl^{-1}) along with IBA (0.2 mgl^{-1}) was found to exhibit highest frequency of shoot multiplication (83 %), maximum number of shoots (13.56 shoots / callus) and shoot length (6.3 cm) which subculturing the leaf derived callus (Table 2). In support of this fact Arya and Shekhawat [11], Cobman and Ernst [12] and Dewan *et al* [13] reported that cytokinins as an obligatory part of the media for shoot differentiation in many plant species. For the induction of roots, *in vitro* grown shoots were transferred to MS medium supplemented with different combinations and concentrations of growth regulators. The effective rooting (74 %) higher number of roots (10.37 roots /shoot) and greater root length (6.9 cm) were obtained by subculturing the *in vitro* developed shoots on the MS medium fortified with the growth regulators IBA and Kn at 2.0 and 0.2 mgl^{-1} respectively (Table 3). Similar findings of the greater rooting performance by higher concentration of auxin in MS medium have been reported in several medicinal plants like *Catharanthus roseus* [14], *Leptadenia reticulate* [15]. The presence of auxin at higher concentration facilitated better rhizogenesis.The requirement of cytokinin (TDZ) for better rooting was reported in many species by various workers [16, 17,18,19,20].

Quint *et al* [21] reported that the rooting response to auxin includes a rapid initial cell growth by changing the pH and calcium, and gene expression which are more effective in the auxins, IBA and NAA combinations rather than the combinations of any other auxins as the inter conversion of these two auxins is more possible and successful, a process required for effective rooting.

After the development of roots, the plantlets were taken out from the culture bottles and washed with sterilized distilled water to remove adhering agar medium, so that the chance of contamination could be stopped. Then these juvenile plantlets were transferred to the hardening medium containing garden soil, sand and vermicompost (1:1:1 ratio by volume) where the leaf callus derived plantlets survivability rate was higher 81% (Table 4). Admixture of all these three components may offer condusive

environment by providing proper nutrients, adequate aeration and required minerals respectively to the plantlets. The present paper describes a prime and easy to use protocol for large scale production of the plantlet *Corchorus acutangulus*.

Table 3. Effect of different concentrations of growth regulators on rooting percentage, root number and root length after the subculturing of leaf derived shoots of the species, *Corchorus acutangulus.*

Growth regulators (mg/L)			Shoots rooted (%)	No. of roots/ shoot	Root length (cm)
IBA	IAA	Kn			
0.5	0.0	0.0	40.36^{b} ± 0.82	4.35^{abc} ± 0.82	3.5^{a-d} ± 0.41
1.0	0.0	0.0	48.79^{d} ± 1.63	4.76^{abc} ± 0.41	2.8^{ab} ± 0.65
1.5	0.0	0.0	52.59^{e} ± 0.82	5.48^{bcd} ± 1.63	3.0^{abc} ± 0.82
2.0	0.0	0.0	57.43^{g} ± 0.41	4.25^{abc} ± 0.82	4.8^{c-f} ± 0.65
2.5	0.0	0.0	60.90^{h} ± 1.63	6.46^{cde} ± 0.82	5.4^{ef} ± 0.33
3.0	0.0	0.0	48.00^{d} ± 0.82	5.56^{bcd} ± 1.63	4.0^{b-e} ± 0.82
0.5	0.0	0.2	44.75^{c} ± 0.65	4.26^{abc} ± 0.41	3.0^{abc} ± 0.82
1.0	0.0	0.2	53.47^{e} ± 1.63	5.38^{bcd} ± 0.82	3.0^{abc} ± 1.63
1.5	0.0	0.2	65.38^{i} ± 0.82	6.76^{cde} ± 0.82	5.0^{a} ± 0.82
2.0	0.0	0.2	74.38^{j} ± 1.63	10.37^{e} ± 1.63	6.9^{f} ± 0.82
2.5	0.0	0.2	67.46^{i} ± 0.82	7.65^{de} ± 0.41	5.0^{def} ± 0.41
3.0	0.0	0.2	56.57^{fg} ± 0.41	5.98^{a} ± 0.82	4.0^{b-e} ± 0.82
0.0	0.3	0.0	31.06^{a} ± 1.63	1.43^{ab} ± 1.63	1.2^{abc} ± 1.63
0.0	0.3	0.0	41.45^{b} ± 0.82	3.23^{ab} ± 0.82	4.2^{b-f} ± 0.16
0.0	0.3	0.0	48.34^{d} ± 1.63	4.49^{abc} ± 1.63	3.1^{abc} ± 0.82
0.0	0.3	0.0	49.64^{d} ± 0.82	5.75^{bcd} ± 0.41	3.4^{a-d} ± 0.33
0.0	0.3	0.0	52.28^{e} ± 1.63	6.00^{cde} ± 1.63	2.5^{ab} ± 0.41
0.0	0.3	0.0	54.17^{ef} ± 0.82	4.37^{abc} ± 0.82	3.2^{a-d} ± 0.16

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT.

Table 4: Effect of different composition of hardening medium on survivability of leaf callus derived plantlets of the species, *Corchorus acutangulus.*

Hardening medium composition (V/V)	No. of plantlets under hardening	No. of plantlets survived	Survivability (%)
Red soil + sand (1:1)	50	22	44^{a} ± 1.63
Garden soil + sand + vermicompost (1:1:1)	50	42	81^{e} ± 0.82
Decomposed coir waste + perlite + compost (1:1:1)	50	36	74^{d} ± 0.41
Vermicompost + soil (1:1)	50	30	70^{c} ± 1.22
Red soil + sand + vermicompost (1:1:1)	50	28	56^{b} ± 0.82

Means in column followed by different letter are significant to each other at 5% level according to DMRT.

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