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Research Article

A RAPID MULTIPLICATION METHOD OF *OLDENLANDIA UMBELLATA* L. - A DYE YIELDING MEDICINAL PLANT

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ABSTRACT

Oldenlandia umbellata L. (Rubiaceae) is an ancient Indian herb used as a source of red dye and various medicinal products. The plant is well-known in Siddha medicine for its styptic property. It is also used as drug for bronchial asthma, febrifuge, to treat poisonous bites and ulcers. These varied uses have increased the utilization and exploitation of this plant for medicinal and dye extraction purposes. Hence, a protocol for rapid propagation is standardized in the present study. The shoot tips and nodes were cultured on MS medium supplemented with different concentrations of BAP and Kin alone or in combination of these two cytokinins ranging from 5-25 µM. The explants growing on medium supplemented with 10 µM BAP was found to induce more number of shoots. In combination with 6 µM Kin further yielded higher shoots of 38 per explants. The developed shoots were transferred to half strength MS medium fortified with IAA, IBA and NAA ranging from 3-15 µM. The best result was achieved on 6 µM IBA. The well rooted plants were transferred to paper cups containing FYM, red soil and sand in the ratio of 1:2:1 for hardening. Then they were transferred to the field for acclimatization. About 85% survival was achieved in the field.

INTRODUCTION

Oldenlandia umbellata L., "Indian Madder", is an important medicinal and dye yielding plant belonging to Rubiaceae. Several members of Rubiaceae are used in traditional medicine (Seydel and Dornenburg 2006). This plant is well-known in Siddha medicine for its styptic property. Both leaves and roots are deemed as good expectorants, and used for treatment of asthma, bronchitis, and bronchial catarrh (Gupta *et al*., 2007). A decoction of leaves is used as a rinse to treat poisonous bites (Rekha *et al*., 2006). A decoction of the root is also used as a febrifuge. Siva *et al*., (2009) reported a novel pH indicator dye from this plant. For toxic bites and ulcers the decoction prepared from its roots and leaves are used as external wash. Sethuramani *et al*., (2014) reported the whole plant extract exhibits significant antitumor activity of Dalton's Lymphoma as cites (DLA) bearing mice. And many studies are being conducted to evaluate the medicinal value of *O. umbellata*.

The root bark of a two-years-old plant, when used with a mordant will conferred color to calico, wool and silk fabrics (Siva, 2007). These varied uses have increased the utilization and exploitation of *O. umbellata* for medicinal and dye extraction purposes. So the present study is aimed to develop a very rapid propagation method for *O. umbellata* through tissue culture.

MATERIALS AND METHOD

Plant material and explant preparation

In this study, *O. umbellata* L. was selected for *in vitro* regeneration from the shoot tip and nodal explants. The plants were collected from Government Arts College Campus, Karur, Tamil Nadu. Shoot tips and nodes excised from healthy plants were used as explants. The explants were washed thoroughly under running tap water for 20 minutes followed by pretreatment with teepol (5% v/v) for 5 minutes. They were rinsed with distilled water for 4-5 times. Then they were disinfected with 70% alcohol (v/v) for 45 seconds and followed by surface sterilized with 0.1% mercuric chloride (w/v) for 3 minutes. Finally, the explants were washed with sterile distilled water for 3-5 times to remove the traces of mercuric chloride.

Culture media and sterilization

MS medium (Murashige and Skoog, 1962) was used as basal medium with 3% sucrose and respective growth regulators for regeneration. The medium was solidified with 0.8% agar. The pH of the medium along with Plant Growth Regulator (PGR) was adjusted to 5.8±0.02 prior to autoclaving at 1.06 kg cm^{-3} at 121ºC under 15 lbs per sq. ft. pressure for about 20 min.

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Shoot induction and proliferation

The shoot tips and nodal explants were cultured on MS medium supplemented with different concentrations of cytokinins, viz., BAP (5-25 µM) and Kin (5-25 µM) either alone or in combination of optimum concentration of BAP along with low concentration of Kin for shoot regeneration and mass proliferation.

Rooting and culture condition

The regenerated shoots of 4 cm and above were transferred to MS basal medium alone and also with different concentrations of IAA, IBA and NAA ranging from 3-15 µM for rooting. All cultures were maintained at 25±2°C in a culture room under cool white fluorescent lamps (Phillips, India) at intensity of 50 $\mu Molm^{-2} s^{-1}$ with 16 hrs photoperiod.

Hardening and Acclimatization

Plantlets with well-developed roots were removed from the culture tubes and washed gently under running tap water to remove the adhering culture medium. The plantlets were transferred to paper cups containing FYM, red soil and sand in the ratio of 1:2:1 and maintained inside the culture room at 25±2°C under 16 hr and 75-80% relative humidity. The plantlets were periodically irrigated with water for a period of two weeks. Subsequently these plantlets were established in the field through acclimatization.

Experimental Design, Data Collection and Statistical Analysis

All the experiments were repeated three times and each experiment consisted of one explant per tubes and five replicates. The different parameters were recorded such as shoot induction frequency, number of shoots per explant, shoot length, number of roots per shoot, root length and survival rate (%).

RESULTS AND DISCUSSION

Shoot Regeneration

The shoot tip and nodal segments were cultured on MS basal medium supplemented with different concentration of BAP (5-25µM) and Kin (5-25µM) alone and in combinations of both the cytokinins. Among the two cytokinins BAP at 10µM concentration gave good noticeable result for both the explants (Fig 1; Table 1).

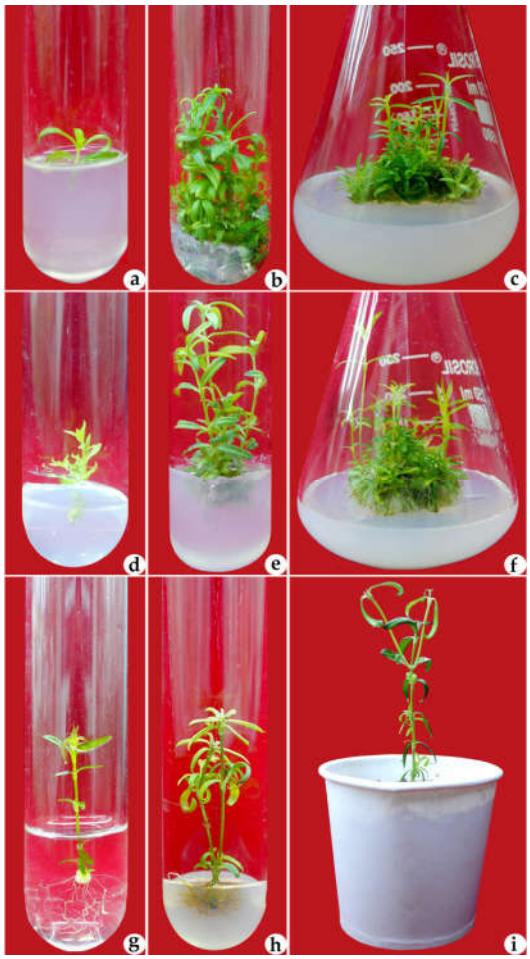

Figure 1 Micropropagation of *Oldenlandia umbellata* L. **a-c**. Shoot induction and proliferation on MS medium from shoot tip; **d-f.** Shoot induction and proliferation on MS medium from node; **g,h.** Root initiation and proliferation; **i.** Hardening.

At this concentration 100% shoot induction frequency with an average number of 38.9 shoots per node and 96% shoot induction frequency with an average number of 32.8 shoots per shoot tip were achieved.

Table 1 Effect of different concentration of Plant Growth Regulators on Shoot induction from Shoot tip and Nodal explants of *Oldenlandia umbellata*.

Plant Growth Regulators		Shoot Induction Frequency (%)		Number of Shoots		Shoot Length (cm)	
BAP	KIN	Shoot tip	Node	Shoot tip	Node	Shoot tip	Node
5 µM	—	86	88	25.1 ± 1.17	32.7 ± 1.52	5.7 ± 1.09	6.1 ± 1.46
10 µM	—	96	100	32.8 ± 1.69	38.9 ± 1.57	7.2 ± 1.19	7.8 ± 1.27
15 µM	—	90	96	31.6 ± 1.11	35.4 ± 1.87	6.4 ± 1.02	6.6 ± 1.22
20 µM	—	82	86	24.1 ± 1.70	27.6 ± 2.24	5.5 ± 1.11	4.7 ± 0.79
25 µM	—	78	74	21.6 ± 1.79	25.5 ± 1.84	3.9 ± 0.84	4.9 ± 1.16
—	5 µM	74	86	21.9 ± 1.81	23.4 ± 1.44	4.4 ± 0.63	4.7 ± 0.90
—	10 µM	88	90	27.8 ± 1.89	28.3 ± 1.43	5.3 ± 1.09	6.2 ± 1.42
—	15 µM	92	98	29.6 ± 1.55	35.1 ± 1.65	6.9 ± 1.06	6.6 ± 0.98
—	20 µM	80	76	20.5 ± 1.81	20.2 ± 1.80	4.4 ± 1.08	5.3 ± 1.03
—	25 µM	66	72	17.6 ± 2.19	19.1 ± 1.31	3.7 ± 1.00	4.3 ± 1.04
10 µM	2 µM	86	94	32.0 ± 1.88	35.1 ± 1.50	5.9 ± 1.21	5.3 ± 0.58
10 µM	4 µM	90	100	36.1 ± 1.57	37.7 ± 2.74	6.7 ± 1.40	7.6 ± 1.21
10 µM	6 µM	100	100	38.7 ± 1.87	46.5 ± 1.41	8.3 ± 1.19	8.7 ± 1.66
10 µM	8 µM	92	94	30.3 ± 1.72	35.4 ± 1.76	6.3 ± 1.18	6.1 ± 1.05
10 µM	10 µM	88	86	27.6 ± 2.31	32.6 ± 1.85	4.2 ± 1.05	4.4 ± 1.08

Values are mean of 5 replicates recorded after 30 days of culture.

Kin at 15µM possessed 98% shoot induction from node and 92% shoot induction from the shoot tip. Combination of these two cytokinins, 10µM BAP with 6µM Kin yielded 100% shoot induction from both the explants with maximum number of 46.5 shoots per node and 38.7 shoots per shoot tip. A number of growth regulators are available for shoot multiplication and proliferation. Among them BAP and Kin are widely used. Many studies also conclude that BAP and Kin had high influence on shoot development for shoot tip and nodal explants (Muhammet *et al*., 2016). Of the two cytokinins tested, BAP was more effective in shoot induction and proliferation than Kin. Similar to this, in several studies BAP was more effective in inducing and sprouting of a large number of shoots (Sahoo and Chand, 1998; Kadota and Niimi, 2003; Velayutham and Ranjithakumari, 2003; Martinussen *et al*., 2004; Velayutham *et al*., 2006; Padmapriya *et al*., 2011; Sweety *et al*., 2016; Manokari *et al*., 2016). Several workers showed that the synergistic combination of two cytokinins was more effective for shoot differentiation (Velayutham *et al*., 2006; Selvaraj *et al*., 2006; Baskaran *et al*., 2008; Sija *et al*., 2016).

Rooting

The well-developed shoots were excised and transferred to half strength MS medium containing different concentrations of IAA, IBA and NAA (3-15 µM) for root induction (Fig 1; Table 2). The roots were initiated on all the concentrations of auxins, but the maximum number of roots per shoot was observed on 6 µM IBA. The length of the root also high at this concentration. Previous studies showed that half strength medium with IBA promote faster the root development (Sweety *et al*., 2016) than full strength MS medium. These results showed that among all auxins, IBA was better in root induction and growth when compared to IAA and NAA (Anis M and faisal M., 2005; Zeping *et al*., 2015) particularly for this plant.

Table 2 Effect of different concentration of auxins on Root induction from the isolated micro shoots of *Oldenlandia umbellata.*

Concentration of Auxins	Root Induction Frequency (%)	Number of Roots	Root Length (cm)
3 µM IAA	82	11.1 ± 1.76	5.0 ± 1.02
6 µM IAA	84	13.8 ± 1.60	5.8 ± 1.41
9 µM IAA	78	9.6 ± 1.30	4.6 ± 0.74
12 µM IAA	70	8.5 ± 2.14	4.2 ± 1.14
15 µM IAA	66	5.4 ± 1.59	3.5 ± 1.09
3 µM IBA	92	17.5 ± 1.15	5.7 ± 1.23
6 µM IBA	100	22.2 ± 1.98	6.5 ± 1.04
9 µM IBA	94	20.1 ± 2.71	6.1 ± 1.00
12 µM IBA	86	16.2 ± 1.73	5.2 ± 1.21
15 µM IBA	76	10.2 ± 1.52	4.6 ± 1.02
3 µM NAA	86	12.4 ± 1.47	3.8 ± 1.04
6 µM NAA	88	15.9 ± 1.12	5.9 ± 1.55
9 µM NAA	96	19.0 ± 1.16	6.1 ± 1.25
12 µM NAA	80	11.1 ± 1.26	4.6 ± 1.05
15 µM NAA	72	8.7 ± 1.51	3.7 ± 1.10

Values are mean of 5 replicates recorded after 30 days of culture.

In the present study, of the three auxins tested, IBA at 6µM concentration was found to induce more number of roots (22.2 ± 1.98) and maximal root length (6.5 ± 1.04) followed by NAA. The results of the present study showed that the developed protocol was more effective for root induction process of *O. umbellata*.

For root induction, IAA, IBA and NAA were used for several studies. Higher frequency of rooting was achieved by IBA in *Aristolochia indica*, (Manjula *et al*., 1997), *Gymnema sylvestris* (Komalavalli and Rao, 2000), *Eclipta alba* (Baskaran and Jayabalan, 2005), *Heliotropium indicum* (Senthilkumar and Rao, 2007), *Melothria maderaspatana* (Baskaran *et al*., 2008), *Ruta graveolens* (Ahmad *et al*., 2010), *Solanum nigrum* (Padmapriya *et al*., 2011) and *Astracantha longifolia* (Senthilkumar and Nandi, 2015). Whereas, NAA showed better root induction in *Cichorium intybus* (Velayutham and Ranjithakumari, 2003), *Plumbago zeylanica* (Velayutham *et al*., 2005), *Hybanthus enneaspermus* (Velayutham *et al*., 2012) and *Enicostemma littorale* (Nalini and Velayutham, 2013), *Hibiscus subdariffa* L. (Manokari *et al*., 2016)

Hardening and Acclimatization

The plantlets with developed roots were transferred to paper cups containing FYM, red soil and sand in the ratio of 1:2:1 and maintained inside the culture room at 25±2°C under 16 hr and 75-80% relative humidity. Subsequently these plantlets were established in the field through acclimatization. Acclimatization is a crucial step to determine the success rate of *in vitro* propagation method. In this study 85% of survival rate was achieved in the field. This indicates the effectiveness of the developed propagation method and this is the first report of *in vitro* propagated *O. umbellata* at field level study.

CONCLUSION

The present study concludes that the combinations of cytokinins have significant effect on shoot induction and proliferation on *O. umbellata*. When compared to shoot tip explants the nodal segments showed good response for all the hormones tested. So nodal explants would be the best choice for rapid multiplication of *O. umbellata*. The influence of IBA on root induction of *O. umbellata* also clearly examined and highest root induction frequency was achieved. About 85% of survival rate was achieved in the field.

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