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SHORT COMMUNICATION

DIRECT RHIZOGENESIS FROM *IN VITRO* LEAVES OF *WITHANIA SOMNIFERA* (L.) DUNAL

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Direct *in vitro* rhizogenesis was induced in *Withania somnifera* (L.) Dunal leaf segments using exogenous addition of auxins. Among the four types of explants (internodal, nodal, young leaf and mature leaf segments), young leaves responded better and formed roots in the midrib region on the contact surface when placed on Murashige and Skoog's (MS) basal medium containing various types (IAA, IBA and NAA) and concentrations of auxins. The strength of the MS media (1/4, 1/2, 3/4 and full-strength) treatments had apparent effect on rooting. Maximum rooting in young leaf (95%) occurred in ½ strength MS + IAA medium. The other types of auxins were good for inducing root in other explants. Only 20 percent of the cultures produced roots if explants were grown on full-strength MS medium supplemented with IBA.

Key words: **Auxins, rhizogenesis, *Withania somnifera***

Withania somnifera (L.) Dunal also known as Aswhagandha, Indian ginseng and winter cherry has been an important herb in the ayurvedic and indigenous medicinal systems for over 3000 years. Historically, its tuber has been used as an aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat bronchitis, asthma, ulcers, emaciation, insomnia and senile dementia. Clinical trial and animal research support the use of Aswhagandha for anxiety, cognitive and neurological disorder, inflammation and Parkinson's disease. It is an ingredient of many formulations prescribed for a variety of musculoskeletal conditions and as general health tonic for elderly persons and lactating mothers (Sangwan *et al.* 2004).

In light of varied pharmacological applications of *W. somnifera* roots, *in vitro* root culture offers several advantageous characteristics over field grown crop. Though, no serious pest has been reported so far in the crop *W. somnifera*, seedling mortality is a major problem which reduces plant population drastically ultimately reducing root yield (Panday 1989). Growth of roots under controlled environmental condition provides easy establishment of axenic culture. This can prevent uprooting of large plants causing no disturbance to naturally existing population. *In vitro* root cultures when established for a desired chemotype will ascertain uniform quality of roots.

Several recent studies have focused on the production of commercially important secondary metabolites from *in vitro* cultures, especially callus and *in vitro* root culture of various plants (Lindsey and Yeoman 1983, Banerjee *et al.* 1993, Gita Rani *et al.* 2003). Though a lot of work has been done on various plants of different genera, there are very limited reports

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on induction and establishment of *in vitro* roots in *W. somnifera* (Gita Rani *et al.* 2003). In this context, the present study has been conducted to standardize the protocol for *in vitro* root induction in *W. somnifera*. Present investigation reports the response of various explants in direct rhizogenesis, to obtain uncontaminated *in vitro* seedlings with a maximum survival rate. Contamination and infection is the main stumbling block in the *ex vitro* germination. In our study, seeds were disinfected in (0.1% w/v) aqueous mercuric chloride ($HgCl_2$) solution for a period ranging from 2 to 10 minutes.

After 20 days of seed germination on MS basal medium, internodal, nodal and young leaf and mature leaf segments were used as explants. Leaves from 20 days and 40 days old seedlings were used as source of young and mature leaf explants, respectively. The effect of various auxins viz., IAA, IBA and NAA on root induction and callus response was observed.

Among various time exposures tested, 8 min. treatment was found to be optimum for seed germination (90%) on both MS basal medium and moistened sterilized cotton. Any further stringent sterilization conditions inhibited seed germination (Plate 1A).

Exogenous additions of auxins (IAA, IBA and NAA) influenced root formation in seedling explants at varied frequencies. Among the three auxins used, the maximum rooting was obtained in IAA treatment from young leaf segments (Table 1). IAA level at 0.75 mg/L brought about the maximum response and produced thick and long roots (Plate 1 B & C). Where as, increase in its concentration (above 1.0 mg/L) resulted in callusing from young leaf segments. Same concentration (0.75 mg/L) of IBA and NAA treatments indicated less and calloid roots with considerable callusing at cut ends of young leaf segments (Table 1). The stimulatory effect of IAA on *in vitro* root induction has also been documented in several other plant species (Nayer and Seeni 2002).

Among the four explants (internodal segment, nodal segment, young leaf segment and mature leaf segment), young leaf segment was highly efficient for direct root induction followed by mature leaf segment and internodal

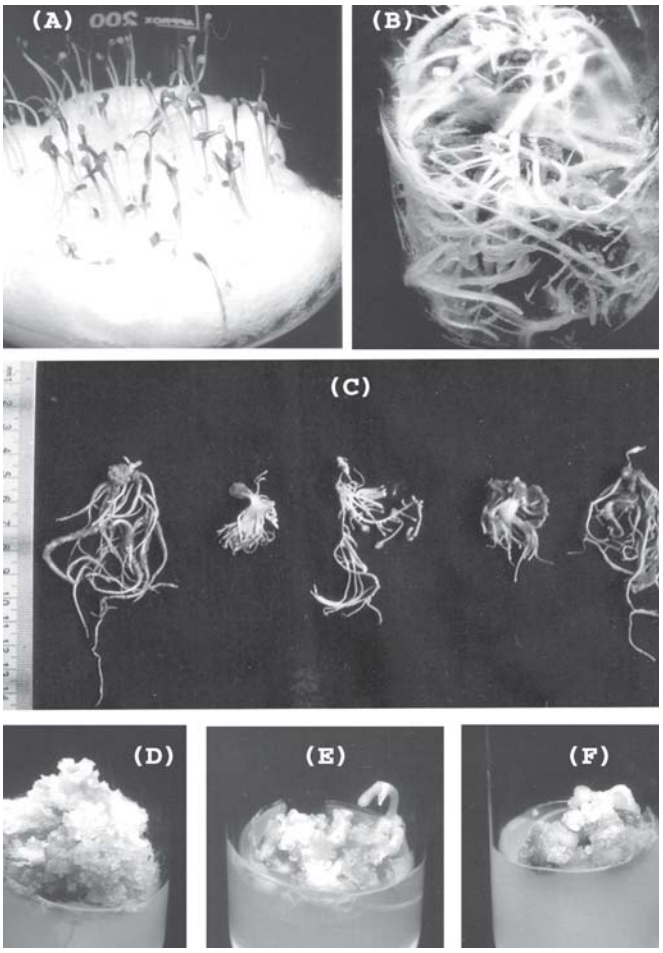

Plate 1. Direct rhizogenesis from *in vitro* leaves of *Withania somnifera*. (A) *In vitro* germinated seedlings of *W. somnifera*, (B) & (C) Directly formed roots on leaf explant, (D), (E) & (F) Callusing response of the explants.

segments. The nodal explants exhibited sprouting of shoot bud along with callus in all three auxins (Plate 1 D, E & F).

Among the cultures incubated under light and dark conditions, an initial dark period of one week and transferring to 12 h photoperiod accelerated both root induction and elongation (Fig. 1). However, prolonged induction in the dark inhibited the *in vitro* root induction. The stimulatory effect of initial dark treatment on root induction is also reported in *Terminalia bellarica* (Roy *et al.* 1987) and *Cleistanthus collinus* (Quraishi *et al.* 1996).

Table 1. Effect of various concentrations of auxins on *in vitro* root induction.

mg/L	Internodal segments			Nodal segments			Young leaf segments			Mature leaf segments		
	% response	No. of roots / explant	Root length	% response	No. of roots / explant	Root length	% response	No. of roots / explant	Root length	% response	No. of roots / explant	Root length
IAA												
0.00	80	-	-	85	-	-	80	-	-	82	-	-
0.25	82	-	-	86	-	-	87	-	-	86	-	-
0.50	87	-	-	89	-	-	92	5±0.7	-	89	-	-
0.75	92	3±0.2	2.8±0.1	93	-	-	95	11±0.3	3.9±0.2	90	4±0.2	2.5±0.5
1.00	95	2±0.5	-	97	-	-	94	8±0.5	2.7±0.4	92	6±0.5	2.9±0.2
2.00	94	Callus	-	92	Callus	-	90	Callus	-	87	Callus	-
3.00	91	Callus	-	90	Callus	-	87	Callus	-	85	Callus	-
IBA												
0.00	82	-	-	85	-	-	87	-	-	85	-	-
0.25	87	-	-	82	-	-	89	-	-	87	-	-
0.50	90	-	-	91	-	-	93	-	-	92	-	-
0.75	92	-	-	95	-	-	95	5±0.3	3.1±0.5	95	3±0.5	2.6±0.3
1.00	95	Callus	-	96	2±0.3	2.1±0.3	97	3±0.7	2.8±0.3	97	2±0.2	2.1±0.5
2.00	93	Callus	-	93	Callus	-	92	Callus	-	92	Callus	-
3.00	91	Callus	-	87	Callus	-	90	Callus	-	90	Callus	-
NAA												
0.00	82	-	-	77	-	-	87	-	-	75	-	-
0.25	87	-	-	80	-	-	90	-	-	80	-	-
0.50	93	-	-	85	-	-	92	-	-	87	-	-
0.75	95	Callus	-	92	Callus	-	95	2.5±0.5	2.5±0.3	90	2.3±2	1.5±0.4
1.00	92	Callus	-	95	Callus	-	93	2.8±0.5	2.1±0.2	92	Callus	-
2.00	90	Callus	-	93	Callus	-	91	Callus	-	87	Callus	-
3.00	83	Callus	-	81	Callus	-	85	Callus	-	82	Callus	-

All the observations were made four weeks after inoculation.

% Response is reported as the behaviour of the explant after inoculation. If root length is not mentioned it should be understood that there is other non-rooting response such as slight callusing or just swelling.

Reduced mineral nutrients in the media are the one of the prerequisite condition for *in vitro* root induction in several plant species (Drews and Van Staden 1995). In the present study reducing MS concentration to half of its strength increase rooting response compared to full concentration (Fig. 2).

The withanolides are the bioactive compounds of *W. somnifera* that are produced in leaf and translocated to root (Abraham *et al.* 1975). In *Agrobacterium* mediated transformation of explants involved major genetic modification of the explants (Ray and Jha 1999). The ability to produce direct roots from leaves can enable to study the production of withanolides in leaf without major genetic intervention. It is also possible to utilize this *in vitro* direct rhizogenesis protocol in metabolic engineering of this medicinal plant (Wadegaonker *et al.* 2006).

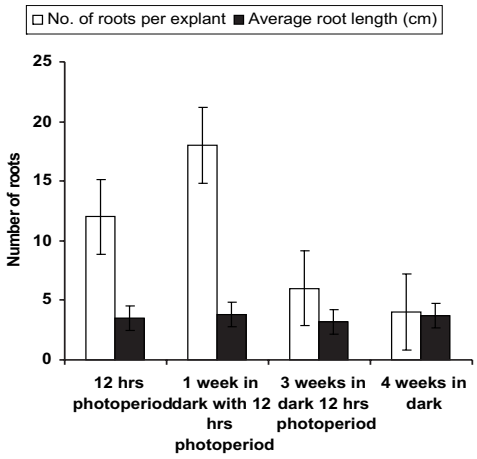

Fig. 1. Influence of dark treatment on *in vitro* root inductions from young leaf segments

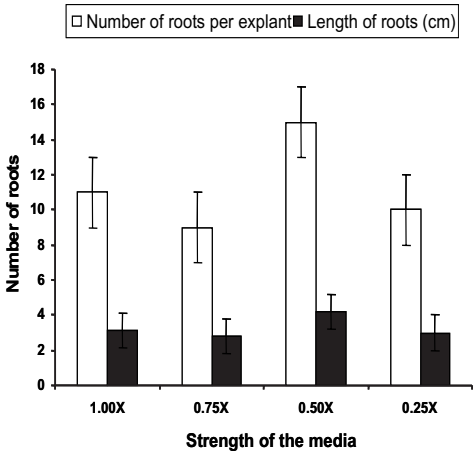

Fig. 2. Influence of MS media concentration on root induction and elongation

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