

Shoot Regeneration from Leaf Explants of *Withania somnifera* (L.) Dunal

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Abstract

Regeneration from leaf explants of *Withania somnifera* (L.) for mass propagation was studied on Murashige and Skoog's medium supplemented with Kinetin (Kn) and 6-benzylaminopurine (BAP) alone or in combination. Shoot buds were induced from the midrib on the abaxial side in presence of Kn and BAP (4 μ M). These shoot buds developed into shoots on the same medium. Rooting of these shoots was achieved in 0.5 μ M of IBA.

Keywords: cytokinin, regeneration, shoot buds

Introduction

Withania somnifera (L.) Dunal belonging to family *Solanaceae* is well known for its medicinal properties and frequently used in traditional system of medicine. It is used in relief of pain, cure of skin disease, diabetes, gastrointestinal disorders, rheumatism, epilepsy and for improvement in general health (Tripathi *et al.*, 1996). This species propagates easily by seeds but does not have the natural ability for regenerative propagation, and hence the variability generated by sexual recombination is difficult to fix (Jayanti and Sharma, 1991). *In vitro* propagation of this useful medicinal plant could provide a means of disease free healthy clones for extraction of pure drugs. This communication reports the morphogenetic capacity of the excised leaves of *W. somnifera* (L.) Dunal.

Materials and methods

Healthy leaves were collected from elite plants of *Withania somnifera* (L.) Dunal from the M. S. University Botanical Garden. First to fourth leaf from the apex of branch in a serial order of development was used as explants. Leaves were washed under running tap water for 1 hour and then with teepol. They were surface sterilized with 0.1% HgCl_2 (w/v) and washed three times with sterile distilled water. The entire leaves were cultured on Murashige and Skoog's (MS) medium (1962) containing sucrose (3%) and supplemented with different concentrations of Kn/BAP (2-6 μ M). They were cultured with their abaxial surface in direct contact with the nutrient medium.

For rooting 4-5 cms shoots were transferred to half strength MS medium containing sucrose (1%) supplemented with various concentrations of IBA (0.5-2 μ M).

The pH of all the media was adjusted to 5.8 and autoclaved at 1.1 kg /cm² (121°C) for 20 minutes. All the cultures were incubated under white fluorescent light (2000 lux) for 16/8 hr light/dark at 25±2°C. In all the experiments 15 replicates were maintained and the experiment was repeated twice.

Results and discussion

First to third leaves when cultured on all test media failed to regenerate any organs and turned white within four weeks. The fourth leaf when cultured on MS basal medium without growth regulators was unable to show any morphogenetic response. In presence of an individual cytokinin Kn / BAP (2-6 μ M) the leaf swelled and remained green for four weeks but failed to differentiate any shoot buds.

The foliar explants responded when Kn along with BAP was added in the medium. In presence of both Kn and BAP (2-6 μ M) the leaves swelled in size and shoot bud induction took place from the midrib present on the lower surface. Lower concentrations of Kn (2 μ M) and BAP (2 μ M) could evoke a poor morphogenic response with the induction of meager number of buds (2.16±0.4). Keeping Kn constant and increasing the BAP level to 4 μ M led to increase in the number of shoot buds. Induction of shoot buds was observed as swelling of the midrib on the lower surface of the lamina followed by formation of greenish protuberances (Fig. 1) from it at the end of two weeks of culture. A maximum number of 12.1±1.2 shoot buds were formed in 90% cultures when both the cytokinins were at 4 μ M/l (Tab. 1). This combination proved to be optimal for induction of shoot buds as further increase in BAP level to 6 μ M/l with the same concentration of Kn decreased

Tab. 1. Effect of cytokinin on leaf explants of *W. somnifera* (Mean ±SE)

Cytokinin (µM)		Average no. of shoot buds/ explant	(%)Response
MS basal medium		-	-
Kn		-	-
2-6		-	-
BAP 2-6		-	-
Kn + BAP			
2	2	2.16±0.4	50
2	4	5.1±0.62	55
2	6	6.1±0.62	62
4	2	7.6±1.2	85
4	4	12.1±1.52	90
4	6	4.0±1.0	70
6	2	-	-
6	4	-	-
6	6	-	-

the number of buds. Similar findings are reported in stem discs of *Curculigo orchioides*, where MS medium fortified with BAP and Kn at 1 mg/l each had a synergistic effect on shoot buds induction from its abaxial surface (Nagesh *et al.*, 2009). Induction of adventitious shoot bud regeneration from leaf discs of neem in presence Kn and BAP has been reported by Ramesh and Padhya (1990). When Kn at 6 µM was combined with different concentrations of BAP failed to induce shoot buds from the explant.

Tab. 2. Effect of IBA on *in vitro* rooting in *W. somnifera* (Mean ±SE)

1/2 MS + IBA (µM)	(%) Rooting	Root number/ shoot	Root morphology
0.5	80	1.66 ± 0.188	A
1.0	60	1.16 ± 0.09	A
1.5	40	2.44 ± 0.417	B
2.0	20	2.83 ± 0.467	C

A = long and stout, B = thick and short, C = very thick

In order to find out the ability for plantlet formation, the shoot buds were detached from the explant and cultured on fresh medium of the same composition. Within two weeks of subculture the shoot buds started to grow into healthy shoots (Fig. 2) and new buds were produced from the base of this cluster by the end of four weeks. Govindraju *et al.*, (2003) have reported direct regeneration from leaf explants for the same plant, but adventitious buds were formed when MS medium was supplemented with BAP (1.0-3.0 mg/l) along with IAA (0.5 mg/l) and Coconut milk (10%). Findings of Sivanesan and Murugesan (2005) have shown that the frequency of shoot bud regeneration varied with the level of plant growth regulators in the medium and the highest frequency of shoot

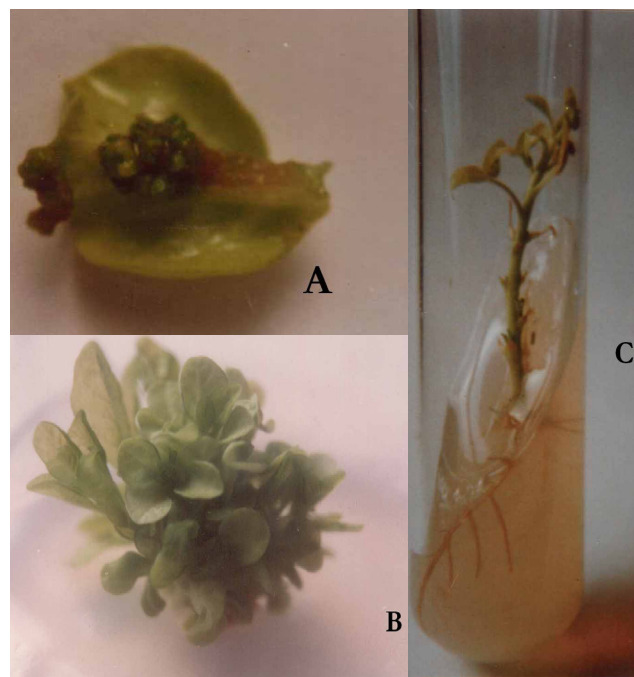


Fig. 1. A) Induction of adventitious buds from abaxial surface of leaf in presence of Kn (4 µM) + BAP (4 µM); B) Further growth of the shoot buds after subculture to fresh medium ;C) Rooting of *W. somnifera* microshoot on half strength MS medium + sucrose (1%) + IBA (0.5 µM)

buds from leaf explant was obtained in presence of only 1.0 mg /l of Kinetin.

In our studies the observations revealed that the explant in presence of individual cytokinin does not regenerate shoot buds. When two cytokinins are incorporated in the medium their synergistic effect induces shoot bud regeneration. Both the cytokinins were essential for this response. Similar results have been observed in leaf explants of *Crataeva nurvula* (Sharma and Padhya, 1989). Vincent *et al.* (1992) have also reported in *Kaempferia galanga* that MS medium supplemented with Kn and BAP together proved synergistic for induction of shoot buds from leaf explants. Sundherson (1998) have reported adventitious shoot buds from excised leaf explants of *Enicostemma axillare* when cultured on MS medium supplemented with BAP.

In vitro rooting was successfully achieved from microshoots of *W. somnifera* with various concentration of IBA (0.5 µM - 2 µM) as shown in Tab. 2.

Initiation of roots was observed after week of transfer of shoots to half strength MS medium containing sucrose (1%) and supplemented with different concentrations of IBA. Long stout roots initiated from the base of the shoots in 80% cultures in presence of 0.5 µM IBA (Fig. 1C). In a similar way, IBA (0.5 µM) induced optimum rooting in individual elongated shoots of *Tylophora indica* obtained from leaf derived callus (Faisal and Anis, 2003). With increase in IBA to 1 µM showed same response as above but

there was a decrease in % response of cultures. For further concentrations the number of roots induced were slightly more but they became thick and short. Sivanesan and Murugesan (2008) for the same plant reported that IBA was efficient in inducing roots (100%) in regenerated shoots obtained from nodal explants.

The rooted plants were hardened in sand:soil (3:1) mixture, and then transferred to greenhouse conditions.

Conclusions

This study provides a protocol for the adventitious shoot bud regeneration from leaf explants of *W. somnifera* for mass propagation. Leaf explants showed higher morphogenetic potential in presence of Kn and BAP each at 4 µM/l.

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