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In-Vitro Mass Propagation of *Withania somnifera* (L.) Dunal

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Abstract

Ashwagandha (*Withania somnifera* L.) Dunal] is an important medicinal plant and a major source of alkaloids and steroids (withanolids), which is regularly used in pharmaceutical industries. Various vegetative parts were studied for its mass propagation through tissue culture technique. Seeds were pretreated with GA3 (50 and 100 mgl^{-1}) for 24 h and 80% germination was achieved. All the explants were taken from *in-vitro* germinated plant. Among the different explants tested, multiple shoot formation was achieved from shoot-tip and nodal explants in MS medium + 0.25, 0.5, and 1.0 mgl^{-1} kinetin. Nodal explants were selected for mass propagation protocol because it formed maximum number of shoots (16.25 shoots per explant) on MS medium + 1mgl^{-1} kinetin after eight weeks of culture. Increase in concentration of kinetin was most effective for callus formation. For further multiplication these shoots were sub-cultured on MS +0.5 mgl^{-1} kinetin. Presence of IAA at 0.5 mgl^{-1} was most effective medium for rooting of *in-vitro* propagated shoots. However, hardening was not achieved for these propagated plants.

Key words: IAA, IBA, NAA, kinetin, *in-vitro* multiplication

Introduction

Withania somnifera (L.) Dunal (Ashwagandha) (Solanaceae) is referred to as Indian ginseng; since it is used in a similar way ginseng is used in traditional Chinese and Korean medicine. It is found in drier parts of India, Afghanistan, Srilanka to Nepal. Regmi (1991) reported its distribution in Nepal in the tropical region of Central and Western Development regions. Its representative collections are also reported from Mustang district of Dhawlagiri zone, at 650 to 1000 m height. It is erect, undershrub, attaining from 1.5 m height; external surface of stem is smooth, yellow and internally white. The leaves are ovate, 5-10 cm long, sub-acute, base narrow to petiole, flowers greenish and umbelliform cyme. Its survival from seed is very poor in natural condition, beside this, exploitation, habitat destruction and collection of root parts for medicinal purpose caused depletion of this plant from its natural habitat. Conventional methods of propagation are time consuming, needs a lot of plant material and it becomes expensive and tedious with a relatively very small output. It can't fulfill the demand of this plant. Hence tissue culture method used in this study is one of the best and reliable method of propagation of this species. The present study is aimed to develop a protocol for mass propagation of *W. somnifera* so that people can cultivate this medicinal plant as a cash crop.

Methodology

Healthy pods (Fig. 2) of *W. somnifera* were selected from the plants followed by removal of seed cover (Fig. 3). These seeds (Fig. 4) were thoroughly cleaned by detergent Tween-20 solution and put under running tap water for about two hours. These seeds were again surface sterilized with 70% ethanol for two minutes followed by 1% sodium hypochlorite for 10 min and finally washed three times with distilled water and inoculated on MS basal medium for germination (Fig. 5). Culture tubes which contained inoculated seeds were placed in the culture room at $25 \pm 2^{o}C$ temperature and 12 to 15 h photoperiod for seed germination. After germination obtaining sterile plants is a prerequisite for further experiments.

The different explants were excised aseptically from *in-vitro* grown seedlings and inoculated on MS basal

medium supplemented with different concentration of phytohormoes (Fig. 6 to 10). From this culture experiment, appropriate combination of MS + phytohormones was identified, in which multiplication was maximum. Finally, the plants growing on *in-vitro* condition were acclimatized on natural condition by using different suitable substrates (Fig. 11).

All inoculations were performed in the laminar airflow chamber. Knives and forceps were flamed before and after each inoculation. The explants were cultured at 25 ± 2°C with 200-300 lux and 12-15 h photoperiod.

For statistical analysis Statistical Package for Social Science (SPSS) version 11.5 was used. All data were analyzed using t-test with a significant level of ≤ 0.5.

Results and Discussion

Seed germination

The seeds were pretreated with GA_3 100mgl^{-1} and 50 mgl^{-1} for 24 h, before cultured on MS basal medium. Eighty percent germination was recorded in both conditions but those seeds which were treated with100 mgl^{-1} GA_3 took less time in germination as compared to those which were treated with 50 mgl^{-1} GA_3. Similarly, the work of Hartman and Kester (1972) also showed that GA_3 was effective to increase the rate of germination and helping in the reduction of time of dormancy.

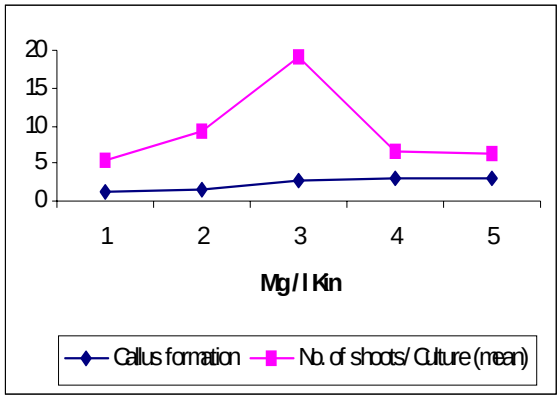
Culture of shoot-tip, node, leaf and root segment

The various concentrations and combination of BAP alone and NAA+BAP were not found as effective as Kin supplemented MS medium for mass propagation protocol.

There was no any difference in shoot-tip and node culture in terms of number of plants produced from each explant. Node culture was selected because it was available in multiple numbers and had less chance of physical injury. Nodal segments were cultured on MS medium supplemented with five different concentrations of Kin (0.25 to 2.0 mgl^{-1}, Table 1).

In case of MS with lower concentration of Kin (0.25 to 1.0 mgl^{-1}, Table 1) light green callus along with healthy multiple (4.0 to 16.25) shoots per culture were formed which is similar to the work of Govindaraju *et al.* (2003), regenerated plants from calli obtained through internodal segments, leaf, petiole explants of *W. somnifera* on MS and B5 media supplemented 2, 4-D (0.5-3.0 mg/l) either alone or with Kin. 0.5 1.0 mg/l. Likewise Sivaneser and Murgeson (2005) obtained highest frequency of shoot bud regeneration of *W. somnifera* on MS+1.0 mgl^{-1} Kin. Varghese *et al.* (1992), obtained maximum number of shoots buds of *Vitex negundo* on medium supplemented with lower concentration of Kin which resembles with the present work.

In case of MS media supplemented with higher concentration of Kin (1.5 to 2.0 mgl^{-1}) the node swelled up and proliferated into light green to light brown coloured callus (fig. 4). From the surface of callus few shoots (3.5 to 3.75 shoots/culture) (fig. 10) were sprouted out after four weeks of culture. These shoots were short, thick and densely covered by narrow leaves. The node and inter-node portions were not distinct. Growths of shoots were also very slow and in tenth week all shoots were turned into pale and yellow colored. This result is dissimilar to the work of Agrawal and Subham (2003) on *Centella asiatica* (L.) observed that 10µm of Kin in basal medium was proved to be optimum concentration for callus differentiation.



Where, 1 = 0.25 mgl^{-1} Kin, 2 = 0.5 mgl^{-1} Kin, 3 = 1 mgl^{-1} Kin, 4 = 1.5 mgl^{-1} Kin, 5 = 2 mgl^{-1} kin.

Fig 1. Graphical representation of effect of different concentration of Kin on node culture.

Table 1. Result of t-test of node culture, comparing number of shoots among different concentrations of Kin, after 8 weeks of culture

SN	Treatments		t-value	Level of significance (p)
A	MS + 0.25 mgl^{-1} Kin	MS + 0.5 mgl^{-1} Kin	-3.962	**0.007**
		MS + 1mgl^{-1} Kin	-4.032	**0.007**
		MS + 1.5 mgl^{-1} Kin	0.655	0.537
		MS + 2 mgl^{-1} Kin	0.333	0.750
B	MS + 0.5 mgl^{-1} Kin	MS + 1mgl^{-1} Kin	-2.814	0.31
		MS + 1.5 mgl^{-1} Kin	6.140	**0.001**
		MS + 2 mgl^{-1} Kin	5.908	**0.001**
C	MS + 1 mgl^{-1} Kin	MS + 1.5 mgl^{-1} Kin	4.295	**0.005**
		MS + 2 mgl^{-1} Kin	4.216	**0.006**
D	MS + 1.5 mgl^{-1} Kin	MS + 2 mgl^{-1} Kin	-0.655	0.537

For significance (P) value must be $\leq$ **0.05**

Form the above fig. 1 shows that increasing the concentration of Kin increases the mass of callus formation. In case of shoots formation increasing the concentration of Kin increases the shoot formation at 1% after which, it decreases shoots formation.

The leaf explants were cultured on full and half strength of MS medium after their treatment with 100 mgl^{-1} BAP, NAA and IAA. Only callus were formed without any organogenesis. Root culture gave no response either in MS or MS supplemented with growth regulators.

The rooting of shoots in IAA at 0.5 mgl^{-1} formed highest number of roots (50 roots per shoot explant). The number of root formation decreased with the change in the concentration of IAA.

Among all these concentrations and combinations of Kin, MS+1.0 mgl^{-1} Kin and MS+0.5 mgl^{-1} Kin mediums were found to be most effective for mass propagation and IAA at 0.5 mgl^{-1} was found to be applicable for rooting of shoots as it formed healthy and maximum number of roots.

For cultivation of *W. somnifera*, poor quality sandy soil with less irrigation was required. High watering leads to death of plantlets as they contain starch and high amount of alkaloids.

Acknowledgements

We would like to thank The Central Department of Botany, Tribhuvan University Kirtipur, Kathmandu for providing opportunity and lab facilities to conduct this research work.

Fig. 1. Plantlet of *W. somnifera*

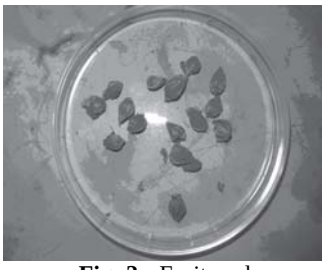


Fig. 2. Fruit pods

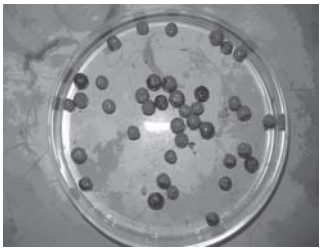


Fig. 3. Fruit capsules after removing of cover

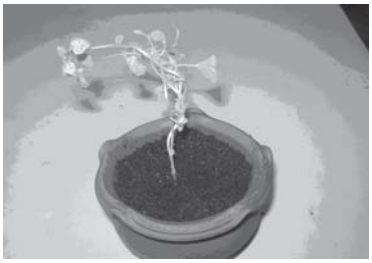


Fig. 11. Acclimatization

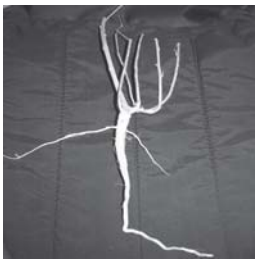


Fig. 12. Root(essential product)

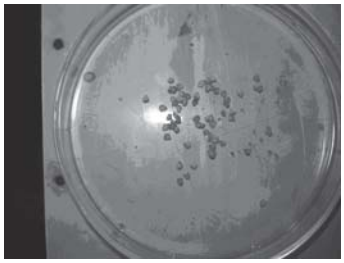


Fig. 4. Seed

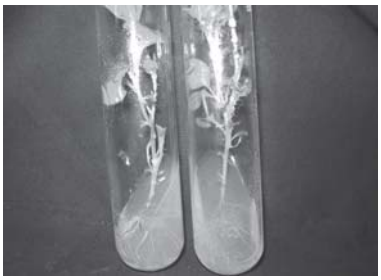


Fig. 10. ***In-vitro*** rooting

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Fig. 5. Seed culture

Fig. 9. Multiplication

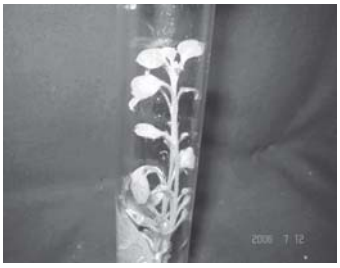

Fig. 6. Seedling

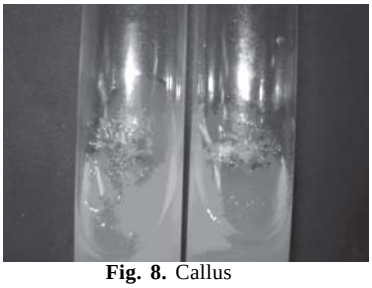

Fig. 8. Callus

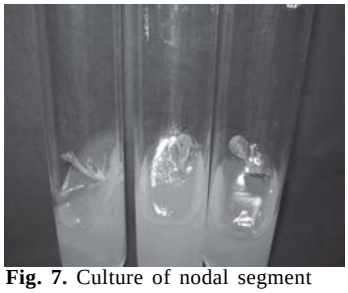

Fig. 7. Culture of nodal segment

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