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RHIZOGENESIS AND CELL CULTURE STUDIES IN *WITHANIA SOMNIFERA* DUNAL

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ABSTRACT:

Rhizogenesis was obtained fromleaf explants Withania somnifera Dunal on MS medium supplemented with different concentrations of auxins supplemented with IAA, IBA, and NAA alone and in combination. The maximum percentage of explant showing root formation and maximum number of roots per explant was noticed on half strength MS medium supplemented with 0.5 mg/l IAA + 1.0mg/l IBA. Cell culture results showed MS+2.0mg/l 2,4-D+0.2mg/l Kin was best liquid medium.

KEYWORDS: *Training, Employee Performance, Perfect, NationLink Telecom Inc.*

INTRODUCTION:

Withania somnifera Dunal belonging from the family solanaceae.In Ayurveda *Withania somnifera* Dunal is widely claimed to have potent aphrodisiac, sedative, rejunative and life prolonging properties. It is used as a general energy enhancing tonic known as Maharasayana. The plant was traditionally used to promote youthful vigour, endurance, strength and health nurturing the time element of the body and increasing the production of vital fluid, blood, lymph, semen and cells. The all plant parts of *Withania somnifera* Dunalhas a medicinal importance.

Generally, roots of *Withania somnifera* Dunal are used in Ayurvedic medicines. The roots are used as a nutrients and health restorative in pregnant women and old man. The decoction of root boiled with milk and ghee is recommended for curing sterility in women. The roots are also used in constipation, senile, debility, rheurration, nervous exhaustion, loss of memory, loss of muscular energy and spermatorrhoea.The chemical constituents of the Ashwagandha are alkaloids and steroids lactones. Among the various alkaloids Withanine is the main constituents. The other alkaloids are somniferinine, withanne, choline, pseudo-withanine, tropine, pseudo-tropine, chlorogenic acid, anahydrine, withanone and the steroidal lactones are withanolides, withaferine and withafenine A present. As the various medicinally important compounds are present in the root, the roots of plants is used in traditional medicine system. In *Withania somnifera* Dunal the roots are the principle source for drug preparation (Kamboj,2000). The development of fast-growing root culture system would offer unique opportunities for producing root drugs in the laboratory without having to depend on field cultivation.

MATERIAL AND METHODS:

Surface sterilized stem. leaves, and nodal explant were cultured on MS nutrient media containing different concentration and combinations of PGRs (Auxins and cytokinesis) to obtain in-vitro cultures. As per requirement, sub-cultures were maintained by sub culturing on fresh medium at 27-30 days of intervals.

RESULTS:

Effect of different concentrations of auxins alone and in combination on leaf ex-plant for Rhizogenesis in *Withania somnifera* Dunal.

The effect of auxins IAA, NAA, and IBA at different concentrations alone and in combination are depicted in table no. 1. For the induction of rhizogenesis in *Withania somnifera* Dunal the leaf ex-plant was cultured on MS medium supplemented with different concentration of auxins (IAA, NAA, IBA) alone and in combination. The leaf ex-plant when cultured on MS medium supplemented with low concentration (1mg/l) of IAA showed rooting and 1 to 2 roots noticed per leaf ex-plant. At higher concentration, of (5mg/l) IAA the leaf ex-plant showed slightly increasing number (4-5) roots per leaf ex-plant and the percentage of ex-plant showing Rhizogenesis was 60%.

Effect of different concentration of auxins in combination for Rhizogenesis.

The effect of different, concentration of auxins in combination for Rhizogenesis are depicted in table no. 1. For the induction of rhizogenesis IAA, NAA, IBA were used in combination of MS medium. The best result for rhizognensis was noticed on half strength MS medium supplemented with 0.5mg/l IAA + 1.0mg/l IBA. On this media 100% ex-plant showed rooting and 12-12 roots are noticed per ex-plant. The lowest response for rhizognensis was noticed on MS + 0.5 mg/l IAA + 2.0 mg/l NAA (Table no.1). Thus, half strength MS media supplemented with 0.5 mg/l IAA + 1.0 mg/l IBA. Was the best medium for the induction of Rhizogenesis in *Withania somnifera* Dunal.

Callogenesis in *Withania somnifera* Dunal

For induction and proliferation of callus the leaf ex-plat was cultured on MS medium supplemented with 2 mg/l 2,4-D + 0.2 mg/l Kinetin. (Gita rani and I.S. Grover 1999) the callus obtained from leaf ex-plant was soft, friable and whitish yellow in colour. The callus was sub cultured on parent media after every three weeks. The maintained callus was used for suspension culture studies.

Suspension cultures of *Withania somnifera* Dunal

The suspension cultures were initiated by transferring 10% w/v of callus into MS medium supplemented with different concentration, of cytokinin kinetin and auxin 2,4-D in combination. The result for suspension culture in different suspension media supplemented with different concentration, of auxin 2,4-D and cytokinin kinetin are depicted in table no.2. The growth of suspension culture in each medium was measured by cell pack volume after 7, 14 and 21 days of cultures. On the basis of cell pack volume measured it was noticed that the best response for cell culture was MS medium supplemented with 2.0 mg/l 2,4-D + 0.2 mg/l Kin. There was gradual increase (0.2, 0.4, 0.6) in cell pack volume from 7 to 14 to 21 days of suspension cultures. Compared to this medium the less response for cell pack volume was noticed on MS + 2.0mg/l 2,4-D + 0.5 mg/l Kin and MS + 2.0 mg/l 2,4_D + 1.0 mg/l Kin.

DISCUSSION:

Most application of plant cell culture in biotechnology are aimed at the production of bioactive secondary metabolites, (Dicosmo and Misawa 1995). Single cell suspension cultures are preferred for large scale production due to its rapid growth cycles. They have been used for generating large number of cells for quantitative and qualitative analysis of growth response and metabolism of novel chemicals.The purpose of the present study was to establish the cell suspension cultures of *Withania somnifera* Dunal.Cell suspension cultures was successfully established by sub-culturing callus into a liquid MS basal medium containing 2.0 mg/l 2,4-D and 0.2 mg/l Kin.

Iranbaksha et.al. (2007) obtained cell suspension from semi clear of leaf ex-plant developed in MS medium containing 0.5 mg/l Kin and 2 mg/l NAA. The present study shows more or less similar results on suspension culture of *Withania somnifera* Dunal compared to these results on *Datura stramonium* which belongs to same family.Nisit et. al. (2007) studies *Capsicum annum* L which belongs to the family solanaceae and obtained cell suspension culture in MS medium supplemented with 2,4-D

and BAP as growth regulators. These results show more or less similarity for the use of auxin 2,4-D in combination with cytokinin for suspension culture.

In many medicinally important plants, the roots are the principle source for drug preparation (Kamboj,2000). The development of fast-growing root culture system would offer unique opportunities for producing root drugs in the laboratory without having to depend on field cultivation (Sudha and Seeni). The present study showed rapid production of roots from leaf explants of *Withania somnifera* Dunal.

Direct rooting from leaf ex-plant of *Withania somnifera* Dunal, was studied by Wadegaonkar et.al. (2005). Maximum number of roots with maximum response was obtained on half strength MS medium supplemented with 2.85 µM IAA and 9.85 µM IBA. More or less similar results for Rhizogenesis are obtained in the present study.

Table -1

Effect of different concentration of Auxin IAA, NAA, IBA alone and in combination on leaf ex-plant for Rhizogenesis of *Withania somnifera* Dunal.

Growth hormone in MS medium	Percentage of explants showing rooting.	No. of roots per explants.
1.0 mg/l IAA	60%	1-2
5.0 mg/l IAA	60%	4-5
2.0 mg/l IAA + 0.5 mg/l NAA	40%	2-3
0.5 mg/l IAA + 2.0 mg/l NAA	40%	1-2
Half MS + 0.5 mg/l IAA + 1.0 mg/l IBA	100%	6-7

Table=2

Effect of different concentration of Auxin 2,4-D in combination with Cytokinin Kinetin on leaf callus for suspension culture of *Withania somnifera* Dunal.

Growth hormone 2,4-D mg/l + Kinetin in MS medium/l	Measurement of growth by cell pack volume		
	After 7 days	After 14 days	After 21 days
2.0 mf/l 2,4-D + 0.5 mg/l Kinetin	0.1	0.3	0.4
2.0 mg/l 2,4-D + 1.0 mg/l Kinetin	0.1	0.2	0.2
2.0 mg/l 2,4-D + 0.2 mg/l Kinetin	0.2	0.4	0.6S

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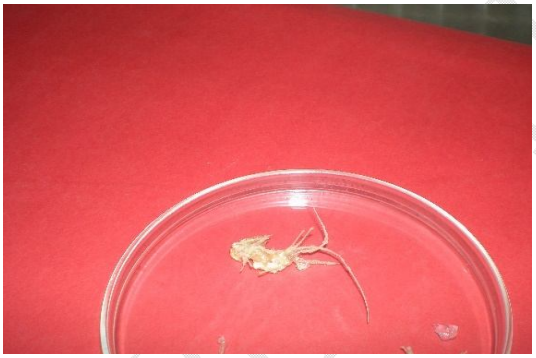
Cell culture after 21 days in MS+2.0mg/l 2,4-D +1.0mg/l Kin.1.0 mg/l IBA

10-12 roots om half MS+0.5mg/l IAA+



4-5 roots/ explant on MS+5.0 mg/l IAA.