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In-vitro Nodal Propagation of Rawolfiaserpentina: An Attempt to Rescue Endangered Plant from Extinction

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Abstract

Rauwolfiaserpentina a medicinal plant is highly exploited for its multi-dimensional application to fight against various ailments and there by being drastically exploited commercially for this reason. The present purpose of this experiment was to develop a cost-effective method to mass propagate the nodal explant through profuse shooting and shoot elongation using growth regulators. A combination of growth regulators were employed in MS media and was observed the best combination of the growth regulators namely auxin and cytokinin was employed(3.0mg/ml Kinetin:0.5 mg/ml Indole-3-butyric Acid:0.5 mg/ml Benzyl aminopurine) supplemented in MS media showed a elongation of 2.8cm in 21 days along with profuse branching at 25 degrees Celsius temperature under artificial light conditions.

Keywords: auxin and cytokinin, cost-effective, mass propagate, multi-dimensional.

Introduction

*Rauwolfiaserpentina*also called Sarpagandha (Sanskrit-meaning smells of serpent), Snakeroot or Devil Pepper belongs to the family Apocynaceae is a perennial shrub plant found mainly in Himalayan regions of India and East Asian countries. This plant has been used as medicines for ages and traced back to Yunani and Ayurvedic medicine. It is used in treatment of Hypertension[1]insomnia, schizophrenia, epilepsy, dyspepsia, used in treatment of insect stings and used as an antidote for snake- bites. It is also used as antipyretic, diuretic, sedative, anti-microbial agent, aids in proper bowl movement, used as anti-helminthic, treatment of ophthalmic and cardio-vascular ailments.[2,3,13] It is containing certain secondary metabolites and alkanoids such as serpentina, reserpine, rescinnamine, ajmalicine etc. mostly indole derivatives. Due to these qualities this plant is exploited immensely by pharmaceutical industries and is in verge of extinction and is kept in red list by International Union for Conservation of Nature. Due to poor rate of germinating seeds due to less viable seed quality and mass exploitation of these wild plants for pharmaceutical purposes and high rate of disappearance our attempt is to aid in preparing plantlets using alternative methods with high rate of propagation using micropropagation technique and conserve this plant species through shooting through nodal explant followed by root initiation using combination of auxins and cytokinins.[4,5,6]

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Material and Method

Explant Preparation:

Young shoots of *Rauwolfiaserpentina* was taken from a year old plant kept in Career College, Bhopal Nursery under experimental conditions required for tissue culture. The explant was washed thoroughly, washed with soap solution then by rinsing with distilled water and surface sterilized using 0.1% $HgCl_2$ solution for 5 minutes followed by washing the explant 4-5 times with distilled water and2cm length explants were prepared containing single node.[7,8]

Media preparation for inoculation of explant:

Explant is inoculated in Murashige and Skoog (MS) media supplemented with growth regulators auxin (Indole-3-acetic acid) and cytokinin (Kinetin/ K; 6-Benzyl aminopurein/ BAP).[6,9] These hormones were introduced into autoclaved MS media in different combinations to prepare test-tubes of varying combinations as seen in Table .1. of K:IBA:BAP respectively.[7]Then the sterilized nodal explants were inoculated individually in respective tubes in laminar air flow and then incubated on plant tissue culture rack and growth and multiplication of shoots were observed for nodal shoot elongation and profuse branching.[10,11,12]

Result and Discussion

The present experiment encompasses the propagation of nodal explant in masses through profuse axillary shoot branching. Surface sterilized explant was initially inoculated in 11 tubes containing varied ratio of growth hormones. It was observed as seen in table 1. That profuse growth was shown by culture RS10 with shoot elongation of 2.8cm on 21 day i.e. 3 weeks having a combination of hormones Auxin (IBA 0.5 mg/ml) and two different Cytokinin (Kinetin- 3.0 mg/ml and BAP-0.5 mg/ml). Upon increasing the auxin and cytokinin concentration a deterioration in profuse branching was seen due to excess of growth hormones resulted in shunting of branching and growing length of the nodal explant.[12,14]

Table 1: Nodal Explant induced upon being inoculated into Murashige and Skoog basal media supplemented with different ratios of AuxinsandCytokinin(Growth Hormones).

S.N.	Culture Name	MS Media Hormones ratio added	Incubation time in days (Growth in cm)		
		K:IBA:BAP (mg/l)	7days	14 days	21days
1.	RS1	0.5:0:0	-	-	-
2.	RS2	0.1:0:0	-	-	-
3.	RS3	0.5:0:0.5	-	-	-
4.	RS4	1.0:0:0.1	-	-	-
5.	RS5	1.5:0:0.5	-	-	-

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6.	RS6	1.5:0:0.5	-	0.1 +	0.2 +
7.	RS7	2.0:0:0.5	-	0.2 +	0.5 +
8.	RS8	2.0:0.1: 0.5	-	0.4 +	0.7 +
9.	RS9	2.5:0.5:0.5	0.2 +	0.5 +	0.7 +
10.	RS10	3.0:0.5 :0.5	0.5 +	1.3 ++++	2.8 +++++
11.	RS11	3.0:0.5 :1,0	0.2 +	0.8 +++	1.0 +++

(-) no growth, (+) growing speed, elongation and number of shoots produced in culture.

Conclusion

It was observed that a combination of auxin and cytokinin was of 3.0mg/ml Kinetin:0.5 mg/ml Indole butyric acid :0.5 mg/ml Benzyl amino purine aided in profuse shooting and elongation of 2.8 cm. higher concentration of these hormone shunted the growth as well as decreased the multiple shoot production from nodes. Different combination of growth regulators may be used to optimize the rooting of these shoots to develop healthy and viable plantlets and aid in rescuing this endangered plant and preventing extinction in the future.

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