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In Vitro Response of GA_3 in Caulogenesis of Fiver nut

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ABSTRACT

A protocol was optimized for the caulogenesis of fiver nut. Internodal explant showed immediate response in shoot regeneration and production of callus in in vitro cultures of Caesalpiniabonducella (L.) Roxb.commonly known as fiver nut. MS medium supplemented with 1 to 10 mg/l GA 3 was found to induce callus. The Internodal explant inoculated on MS medium with 6 mg/l GA3 was found to produce shoots after 35 days of inoculation. Maximum amount of pale yellow coloured friable callus was produced in 7mg/l GA3 of dry weight 1.513± 0.108 g. The method can be used to generate callus and shoot which are natural sources of pharmaceutical compounds without disturbing the natural population of the plant.

Keywords : Fiver Nut, Caulogenesis , Callus.

I. INTRODUCTION

Caesalpiniabonducella (L.)Roxb.is an important medicinal plant belonging to the family Caesalpiniaceae .The entire plant and plant parts like seed ,seed coat , pod ,leaf , stem and roots are used in traditional medicines of India and all over the world. The plant contains biologically active compounds like phenols ,diterpenes , flavonoids ,alkaloids and tannins . Caesalpiniabonducella(L.) Roxb.reported to possess anti-diabetic (SudeepParameshwar et al., 2002) ,anti-filarial (Gaur et al., 2008.),anti- malarial(Sachan NK et al., 2010)and antioxidant (Ogunlana et al., 2012) properties. It is a major constituent of Ayush -64 used against malaria and Female Health by Planet Ayurveda as a supplement to vitamin C . Seed powder is packed and sold under commercial name Kalarchi Kai powder to treat fever. In Ayurveda the plant is reported to balance tri doshas like vata, pitta and kaphadoshas. Unfortunately for this reason the natural habitats of Caesalpiniabonducella (L.) Roxb.were encroached by human beings .The population of fiver nut is drastically reducing for the past two decades. Recently the plant is placed under endangered category and will extinct if steps are not taken for conservation.

Tissue culture is an efficient tool to increase the number of plants of Caesalpiniabonducella. Santosh Kumar et al. carried out micro propagation of fiver nut. In vitro plant regeneration and acclimatization of plantlet can be done to supply sufficient plants to farmers, environmentalists and common man. It will initiate rapid propagation of fiver nut and we can restore natural population. Even thoughauxins andcytokinins are mostly used for shoot regeneration and callus production of fiver nut GA_3 is insufficiently utilized. GA_3 is a chemical derivative of gibberllins naturally present in plants.GA_3 has an impact on stem elongation ,organization of shoot primordia and breaking seed dormancy.GA_3 is used to induce callus and shoot regeneration in woody climbers like grapes. Exogenous supply of GA_3 increases the root auxin in transgenic plants .It is also reported to elevate the production of phytogibberllin content in both root and shoot. Therefore our study

aims to conserve the fiver nut plant by providing (1) an alternative source for the production of pharmaceutical compounds through callus (2) an efficient protocol for caulogenesis in fiver nut with GA_3 which is rarely used in in vitro cultures of Caesalpiniabonducella(L.) Roxb.

II. METHODSAND MATERIAL

The plants and the mature seeds of Caesalpiniabonducella(L.)Roxb.were collected from Manjri and authenticated in BSI. The seeds were washed thoroughly and dried. Seeds were sacrificed using Con. HCl for 60 minutes and it was allowed to germinate. After 10 days epicotyl protruded above the soil .Healthy seedlings were established in one month. Three months old seedlings were used as explant source for the experiment.

Leaf, internode and node were used for in vitro cultures. The explant were washed for 30 minutes in running tap water. Then labolene treatment was given for 5 minutes. The treated explant were washed thoroughly. They were kept again in running tap water for 30 minutes. Then it was treated with 1% $HgCl_2$ followed by three times wash using double distilled water in laminar air flow chamber. About 1cm length explants were inoculated in MS medium supplemented with GA_3 1 to 10 mg/l. After inoculation the culture were kept in culture room with 25±1^{0}c temperature, 16 hours 1000 lux light and humidity 50%. Observations were taken after 5, 10 and 15 days. Sub culturing was carried out at 15 days interval. Fresh weight of callus were taken after 35 days using electronic balance. MS basal medium supplemented with 30g sucrose was used as control for the experiments. The pH of the medium was adjusted at 5.8. Statistical analysis were carried out using ANOVA.

III. RESULTS AND DISCUSSION

Phenolic exudation and browning of cut surfaces of explants were reduced significantly in explants collected from three months old seedlings. The shoot was obtained from inter nodal explants after 35 days of inoculation. Subculture of callus and shoots were done at 15 days interval. Green nodular callus was produced in 6 mg/l GA_3 after 15 days of inoculation and shoot after 35 days. Internodal explants produced callus in MS medium supplemented with GA_3 1to 10 mg/l. Callus was initiated in 10days of inoculation in 1mg/l to 4mg/l GA_3.Maximum amount of callus was obtained in 7mg/l GA_3 with dry weight 1.513±0.108g. Beyond 8 mg/l the callus weight was decreased.

Effect of GA_3 in callogenesis and caulogenesis in Caesalpiniabonducella (L.) Roxb.after 35 days.

Table 1

Hormonal Con.(mg/l) GA_3	Morphology of Callus Color Nature	Amount of callus (Dry Wt. in g) Mean ±SE	No. of shoots
1	Green Friable	0.426 ± 0.059	0
2	Green Friable	0.533 ± 0.033	0
3	Green Friable	0.762 ± 0.034	0
4	Green Friable	0.849 ± 0.042	0
5	Greenish yellow Flexible	0.773 ± 0.045	1
6	Greenish Nodular	0.842 ± 0.034	1

	yellow		
7	Pale yellow Friable	1.513 ± 0.108	1
8	Pale yellow Friable	0.654 ± 0.078	0
9	Yellow Compact	0.618 ± 0.248	0
10	Yellow Compact	0.211 ± 0.145	0

Caulogenesis inGA_3 6mg/l.

A. B.

Callus in GA_3 7mg/l Callus initiation in 7 mg/l GA_3

Callus in GA_3 8mg/l Callus initiation in GA_3 4 mg/l

Callus in GA_3 5 mg/l Callus initiation in GA_3 3mg/l

Caesalpiniabonducella (L.) Roxb.requires 7 years for seed germination which is one of the reason for its current status in endangered medicinal plant in nature. Many researchers worked on in vitro propagation of fiver nut using cotyledonary, epicotyledonary and nodal explants (Santosh Kumar et al.,2012).GA_3 is widely used for the micropropagation of woody trees and climbers(Jain et al.,2007).A combination of GA_3 and cytokinins were used for shoot multiplication in tea(Reza et al.,2014) and Lavenduladentata,L. shoot tips (Marilia et al.,2011).To conclude probably first time GA_3 was used in in vitro cultures of fiver nut for the production of callus and shoot. The regenerated shoot can be used for the extraction of bioactive compounds which is very important to conserve the in vivo plants.

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