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Analysis of anthraquinone by callus tissue of *Aloe barbadensis*

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Abstract
Aloe barbadensis, the miracle plant, is the most widely cultivated species. *Aloe-barbadensis* have been widely used for medicines and cosmetics. Micropropagation studies were carried out in plant tissue culture laboratory, Department of Biotechnology, St. Thomas College, Bhilai. Murashige and Skoog media enriched with IAA, 2,4-D, NAA and Kin was studied. Leaf disc of *Aloe barbadensis* were used as explant. Callus cultured in 2,4-D and Kin shows maximum growth rate and highest AQ production while NAA and IAA shows poor. After 60 days it was found that callus cultured on 2,4-D (3 mgl^{-1}) and Kin (1 mg^{-1}) gives 8.4 µg/gm AQ however, NAA (3 mg^{-1}) and Kin (1 mg^{-1}) gives 5.7 µg/gm.

Keywords: *Aloe barbadensis*, Micropropagation, Anthraquinone.

INTRODUCTION

Aloe barbadensis, probably the most widely cultivated species of the genus aloe in the world in a medium to large shrub that is usually multistemmed at or near ground level. *Aloe barbadensis* have been widely used for medicines and cosmetics and their chemical constituent have been studied[1]. *Aloe barbadensis* mill is one of the important contributions of ancient Indian medicines to the world health care system. It is commonly known as "Korphad"[2]. It is cultivated throughout in the world, both as a household and for its medicinal quantities. Aloe genus comprises about 200 species, indigenous to East and South Africa. Many species are cultivated through out the India, *Aloe barbadensis*,

More than 160 secondary metabolites found in *Aloe barbadensis* leaves. Among these most important metabolites are barbaloin and homonataloin[3]. They are anthraquinone-producing plants and the content of anthraquinone is subject to seasonal variation[4]. The barbaloin content of Aloes was found to be as low as 4.24%.

Aloe barbadensis is used internally as laxatives, antihelminthic, hemorrhoid remedy and uterine stimulant as menstrual regulator and being used for wound healing, stomachic, emmerenjogic, astrigent, antihelminthic conjunctivitis and as a disinfectant and laxatives[5-7]. The Ayuervedic drug known as "Kuamri asava" is very useful in general disability, cough, asthma, piles, epilepsy and colic. It has also show remarkable results on sun damaged skin and UV damaged skin[8].

Cultivation of medicinal plants especially high value medicinal plants is creating new dimension in the field of agriculture. The medicinal plants industry puts together the various facts of multidisciplinary industry and its global interest. This plant generally grows on lower as well as higher altitudes in rare so to conserve this medicinal plant, it should be propagated on lower altitudes to have its commercially significant products like secondary metabolites .For this tissue culture is appropriate technique. Secondary metabolites of *Aloe barbadensis* have got potential application as therapeutics during past few years considerable attention has been focused on the use of plant cell cultures as a source of secondary metabolites.

Secondary metabolites are natural plant products that do not necessarily appear in primary biochemical activities of plant growth development and reproduction. In many plant tissue culture system the quantitative significance of synthesis of secondary metabolites is low. As a consequence, the most impact of the synthesis of these products on cell metabolism is difficult to study; however, few types of cell are able to perform such biosynthesis reaction at high rate, resulting in concentration more than 10 % on dry weight basis.

MATERIALS AND METHODS

Aloe barbadensis was collected from the St. Thomas College, Bhilai. Collected plants of *Aloe barbadensis* were maintained and multiplied in the earthen pots in the controlled conditions. Precautions were taken to avoid any type of trace as well as infections.

Leaf bit explants were selected from these healthy and well-grown plants. The explants washed in running tap water for 10 min and then wash thoroughly with sterilized double distilled water. Explants were dipped in 70 % ethyl alcohol for 30 seconds and all explants were treated with 0.1 % freshly prepared $HgCl_2$ for 2-3 minutes then washed with sterilized double distilled water for three times to remove excess $HgCl_2$ under aseptic conditions.

The medium developed by Murashige & Skoog's (1962)[9] was used with addition of other desired supplements and various combinations and concentrations of plant growth substances with 3% sucrose and 0.25 % phytagel. The surface sterilized leaf bit explants was inoculated on the above medium under aseptic condition. All the inoculated cultures of the *Aloe- barbadensis* were incubated in the culture racks present in the dust free culture room. The temperature was maintained at 27° $\pm$ 2° C. The cultures were kept in light for 16 hrs (2000 – 2500 lux) and 8 hrs dark respectively. The callus was

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cultured using a combination of auxins and Kin as shown in (Table1) and growth of callus was calculated in terms of fresh and dry weight.

The callus (1 gm) was removed from the culture medium and then subjected to extraction in acetonitrile solution (4 ml) by centrifugation at 1500 rpm for 5 min and supernatant was collected and pellet was again extracted with 1 ml acetonitrile and centrifuged (1500rpm) and supernatant was collected and mixed it. The solution was filtered, concentrated and dried in vacuum. The residue was subjected to test the presence of secondary metabolites. The presence of AQ was detected at 220 nm.

RESULTS AND DISCUSSION

Natali *et al.,* 1990 has studied the various auxins ans cytokinins on induction of callus of *Aloe barbadensis*[10]. They reported that 2,4-D and Kin was the best combination for induction of callus. Similar results were obtained by Roy and Sarkar, 1991[11] on medium containing 1 mg^{-1} 2,4-D and 0.2 mg^{-1} Kin. Here in this case, the fresh weight (80 mg) of callus was obtained after 30 days of culture and fresh weight of callus was increased after 90 mg after 60 days of culture in hormone free medium. The callus cultured in medium containing 1-3 mg^{-1} increased fresh weight of callus. The MS medium supplemented with NAA in combination with Kin increase in fresh weight of callus. The 3 mg^{-1} NAA and 1 mg^{-1} Kin gives 480 mg fresh weight of callus. Callus cultured on different combination of IAA and kin gives poor results **(**Fig. 2B) as compare to NAA and 2, 4-D. The 1-mg^{-1} 2,4-D with 1 mg^{-1} Kin gives 530 mg/gm of callus(Fig.2A**)** while 3-mg^{-1} 2,4-D and 1 mg^{-1} Kin contains 844 mg fresh weight of callus(Table1).

The yield of AQ from callus cultured in medium containing different combination of auxin and Kin was different as shown in (Fig. 1). 2, 4-D was found to be most suitable from AQ production (840 µg/gm). The 3-mg^{-1}2,4-D with 1 mg^{-1} Kin give maximum concentration of AQ while 1$mg^{-1}$and 2 mg^{-1} 2, 4-D with 1 mg^{-1} Kin gives 500 and 38 µg /gm AQ respectively. However, callus cultured on medium containing 3 mg^{-1} NAA and 1 mg^{-1} Kin gives 570 µg /gm and 2 mg^{-1} NAA with combination of Kin gives 342 µg/gm of AQ while exogenous IAA had no great effect on cell growth or production of AQ. The 3 mg^{-1} IAA and 1 mg^{-1} Kin give maximum AQ yield (21µg/gm) as compared to other combination of IAA. Therefore the effect of Kin on growth and AQ production id thought to be reflected (Fig.2C**).**

It is well known that pigment formation in tissue culture of *Morinda citrifolia*[12] is inhibited by addition of 2,4-D on the other hand it has been reported that polyketide formation in tissue culture is not affected by 2,4-D[13]. The effect of auxin in our experiment was similar to that of auxin on the formation of AQ, which are derived from polyketide. Sato *et al. (*1991) studied that highest concentration of IAA showed the maximum growth rate and highest AQ production with combination of Kin[14]. Generally the production of secondary compound by plant cell culture is increased under controlled in illuminated condition[15].

Table 1. Effect of auxins and Kin on growth after 2-month-old callus culture of *Aloe barbadensis*

Auxin	Mg^{-1}	Kin(mg^{-1})	Fresh wt. after 30 days (S.D)	Fresh wt. After 60 days (S.D)
	0	0	80 ± 0.9	90 ± 0.4
NAA	1	1	222 ± 0.6	270± 0.9
	2	1	320 ± 1.8	390 ± 0.9
	3	1	416 ± 0.7	480 ± 1.4
2,4-D	1	1	490 ± 1.2	530 ± 0.7
	2	1	780 ± 0.3	844 ± 0.3
	3	1	853 ±0.6	956 ± 0.6
IAA	1	1	98 ± 0.3	110 ± 0.5
	2	1	198 ± 0.7	210 ± 1.2
	3	1	213 ± 0.5	270 ± 0.1

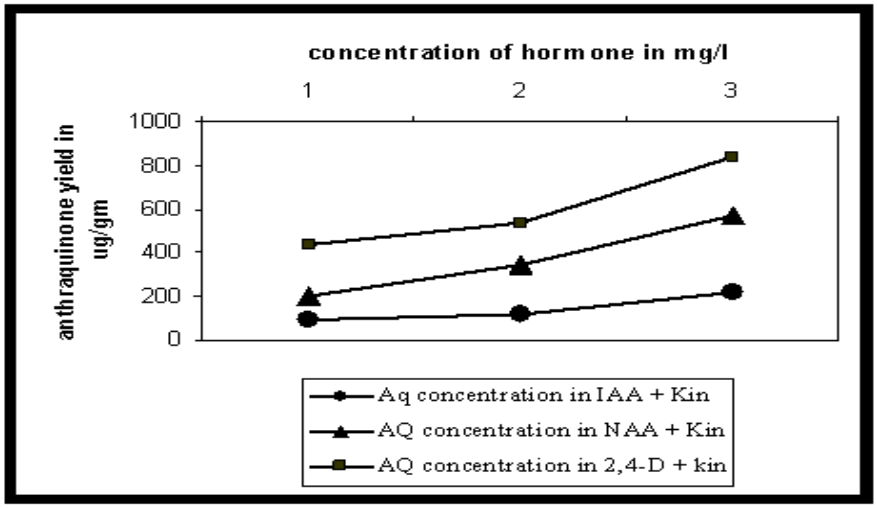

Fig 1. Effect of phytohormones on anthraquinone yield

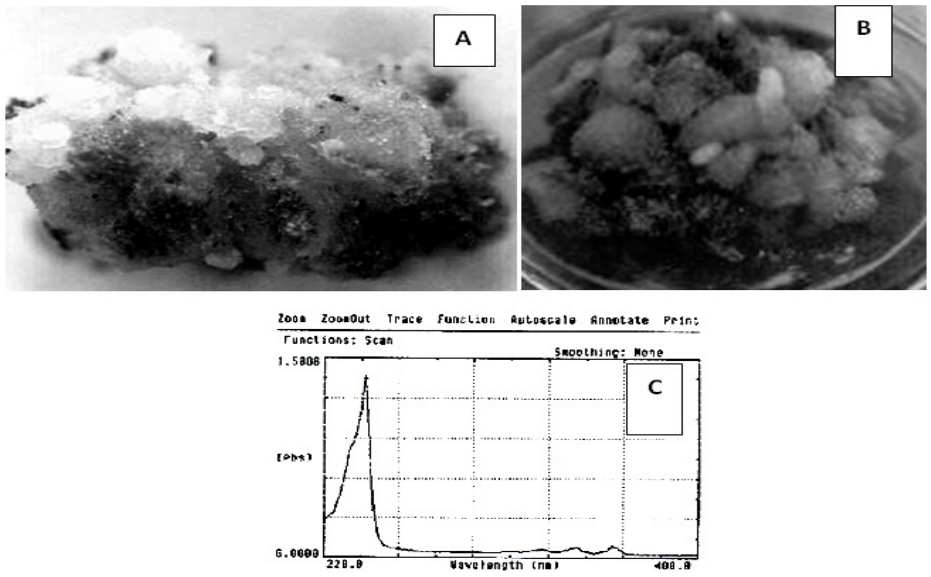

Fig 2. A- Callus induction from 2,4-D with Kin, B- matured callus from IAA with Kin, C- Spectrophotomertic analysis of anthraquinone from callus induced in 2,4-D and Kin .

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