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In vitro Regeneration of *Aloe barbadensis*

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Abstract: Tissue culture has been established as a useful *ex situ* conservation strategy for Aloe species. This paper describes *in vitro* regeneration of *Aloe barbadensis* (Liliaceae) and preliminary estimation of secondary metabolites in methanolic extract of *in vitro* regenerated plants. Shoot tips were used as explants for *in vitro* regeneration on MS medium supplemented with 4 mg L^{-1} Benzyl amono purine (BA) and 1 mg L^{-1} Indole acetic acid (IAA). *In Vitro* rooting was induced when Murashige and Skoog's (MS) medium was supplemented with 1 mg L^{-1} Indole Butyric Acid (IBA). Thin layer chromatography studies were carried out on methanolic extract of *in vitro* regenerated plants.

Key words: *In vitro* regeneration, *Aloe barbadensis*, secondary metabolites

INTRODUCTION

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.

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 (Ross, 1999). 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 (Nakagomi *et al*., 1983; Takayuki and Toshifumi, 1983; Groom and Keynolds, 1987). They are anthraquinone-producing plants and the content of anthraquinone is subject to seasonal variation (Gutterman and Chauser-Volfson, 2000). 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 Lawrence (1984), Chitra *et al*. (1998) and Choi *et al*. (2001). 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 (Afzal *et al*., 1991; Bunyapraphjatsara *et al*., 1996; Mamtle *et al*., 2001).

The uncontrolled collection and sale of large quantities of *Aloe barbadensis* plant material lead to destruction of *Aloe barbadensis* plants from many forests. Local communities, traditional medicinal herbalists and herbal medicine vendors popularly collect Aloe leaves, which is a serious problem.

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 and during past few years considerable attention has been focused on the use of plant cell cultures as a source of secondary metabolites.

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.

Shoot tip explants 1-2 cm 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 sec and all explants were treated with 0.1% freshly prepared $HgCl_2$ for 2-3 min then washed with sterile double distilled water for three times to remove excess $HgCl_2$ under aseptic conditions.

The medium developed by Murashige and Skoog's (1962) 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 shoot tip 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±2°C. The cultures were kept in light for 16 h (2000-2500 lux) and 8 h dark, respectively (Hartman *et al*., 2002).

The number of shoots was examined after 4 weeks of incubation. MS medium of half and full strength and supplementation with IBA (1 mg L^{-1}) was used for rooting. The cultures were incubated under similar conditions.

The *in vitro* regenerated plants were removed from the culture medium and then subjected to methanolic extraction for 8 h in soxhlet extractor. The solution was filtered and concentrated and dried in vacuum. The residue was subjected to test the presence of secondary metabolites. TLC studies for the extract was carried out by using ethyl acetate: methanol: water (100:13.5:10) as mobile phase on silica gel precoated plates using standards. The area corresponding to secondary metabolite anthraquinone was scraped and dissolved in 15 mL methanol. The estimation of anthraquinone in all the samples was done by studying the absorption spectra, ranging from 220-400 nm was directly proportional to the total anthraquinone content in the sample.

RESULTS AND DISCUSSION

Choudhary and Mukundan (2001) reported that in Aloe vera formation of multiple shoots *in vitro* was a function of cytokinin and auxin. Shoot tip segments transferred on MS basal medium produced slow growth compared to the medium supplemented with BAP (4 mg L^{-1}) and IAA (1 mg L^{-1}). Time required for the production of shoot and root portion was 10 days. MS medium with growth hormones produced shoots within three days of transfer however medium with no growth regulator produced shoots after 6 days. Average number of shoot produced on MS medium after 4 weeks of transfer was 9.4 and medium supplement with hormones was 22.4 (Fig. 1) Further proliferation on MS medium with BAP (4 mg L^{-1}) and IAA (1 mg L^{-1}) indicate that it is a good combination of medium for producing multiple shoots from single explants. Aggrawal and Barna (2004) reported that Kinetin and BA promoted shoot proliferation but BA proved better. Chukwujekwu *et al*. (2003) who got maximum shoots at the concentration of BA 1.0-3.0 mg L^{-1}. Further this was also confirmed with

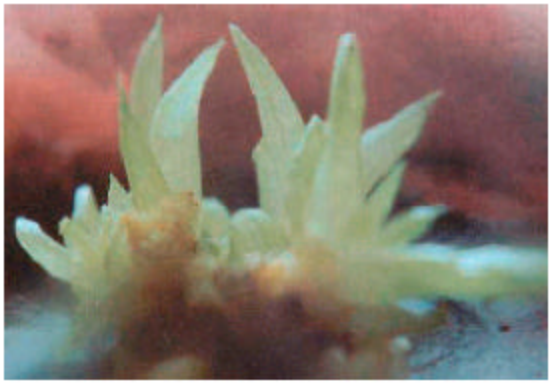

Fig. 1: Shoot regeneration of *Aloe barbadensis* on MS medium with BAP (4 mg L^{-1}) and IAA (1 mg L^{-1}) after 10 days

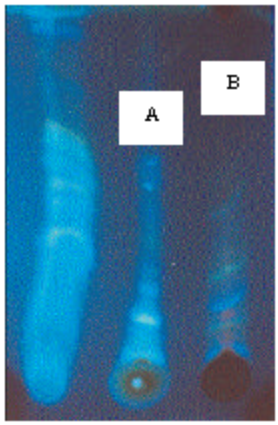

Fig. 2: TLC of standard Anthraquinone and isolated Anthraquinone from *in vitro* regenerated shoots. A: Standard Anthraquinone and B: Isolated Anthraquinone from *in vitro* regenerated shoots

the results given by Meyer and Staden (1991) who reported that high rate of shoot proliferation were obtained from axillary and apical bud of *Aloe barbadensis* when cultured on MS medium supplemented with 0.18 mg L^{-1} IAA and 2.25 mg L^{-1} BA.

Induction of rooting on MS medium of half and full strength and supplementation with IAA (1 mg L^{-1}) indicate that IAA was helpful in increasing root induction and number compared to MS basal medium. Similar results were given by Agrawal and Barna (2004) who showed that maximum rooting with 5.0 roots/shoot was recorded on medium containing 1 mg L^{-1} BA and 0.2 mg L^{-1} IBA. The percentage induction was to an extent of 85% in IAA however 60% root induction was observed in MS basal medium. Medium supplemented with IAA could produce twice the number of rootlets compare to half strength MS medium after 4 weeks of incubation. An average 12

rootlets were produced on MS medium supplemented with IAA where as 5.8 roots were produced in MS basal medium. Rooting was achieved on MS medium with 0.18 mg L^{-1} IAA with 0.2 mg L^{-1} BA followed by MS medium with 0.02 mg L^{-1} IBA (Hirimburegama and Gamage, 1995).

Thin layer chromatography using mobile phase as ethyl acetate: methanol: water (100:13.5:10) gives best results for analysis of Anthraquinone which is similar to Volfson *et al*. (2002). TLC analysis of methanolic extract produced spots with Rf values similar to reference standards Anthraquinone. Sato *et al*. (1990) also extracted Anthraquinone using methanol as solvent. Rf values for anthraquinone was found to be 0.43 and spots in the extracts also shows similar bands (Fig. 2). Anthraquinone appeared yellow band under UV light during preparative studies. The UV scanning of the methanolic solution of the separated Anthraquinone showed at 240 nm Linus *et al*. (1990)and this absorption maxima was similar to that of standards however Zenk *et al*. (1975) obtained absorption maxima for Anthraquinone at 434 nm.

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