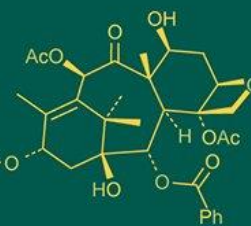
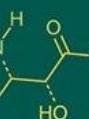
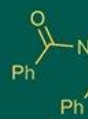


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Effects of different growth hormones on shoot initiation of Vekhand (*Acorus calamus* L.) by using rhizomes

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Abstract

The present investigation has been conducted on “*In-vitro* propagation of Vekhand (*Acorus calamus*) using rhizomes” explants to identify suitable media combinations for shoot initiation of Vekhand. The mother plants of Vekhand were collected from Dhanwantari Nursery, MPKV, Rahuri, Tal: Maharashtra. Rhizome explants were excised from mother stock maintained in shade house. The explants were initiated on MS media with different concentration of BAP ranging from 1.0 mg/l to 3.0 mg/l BAP. Best shoot growth was observed on MS media containing 2.5 mg/l BAP alone. Therefore, 2.5 mg/l BAP was tested along with other hormonal combination to check for the best shoot growth. 2.5 mg/l BAP along with 1.0 mg/l IAA was found to be the best for significant shoot growth.

Keywords: *Acorus calamus*, Invitro propagation, Auxins, Medicinal plant, acclimatization

1. Introduction

The perennial herb known as the sweet flag, *Acorus calamus* L (also known as Bach), is a member of the Araceae family. On the northern temperate, subtropical, and hot regions of the Indian subcontinent, it can be seen in swampy soil, shallow water, and lake edges. Starch, resin (2.5%), volatile oil (1.5-3.5%), and tannins (1.5%) are all present. Asarone, one of *calamus* oil's primary ingredients. *Acorus calamus* is said to have antibacterial and antifungal properties in both its leaves and tuber. There has been a demand for the plant because of its many uses. Rarely does the herb yield seeds; instead, it is typically replicated vegetatively. The medical industry and the preservation of germplasm would both profit significantly from the *in vitro* techniques of clonal reproduction of *Acorus calamus*. Currently, using tissue culture technologies, micropropagation is a crucial assist in rapid clonal replication (Altaf *et al.*, 2010) [1]. The *Acorus calamus* rhizome's crude extract is utilised in drinks and cosmetics. Seed production is scanty in this herb; hence, rhizomes are largely used as propagule for its traditional cultivation, which has a slow rate of micropropagation. As a result, *in vitro* procedures may offer a quicker method of mass micropropagation for the creation of high-quality, disease-free quality seeds as well as a way to conserve genetic material (Quraishi *et al.*, 2017) [7]. Conventional methods of micropropagating this plant is insufficient to supply the plant material needed for large-scale growth and to satisfy the expanding demand of the pharmaceutical companies, according to Hettiarchil *et al* (1997). As a result, new methods for micropropagating this plant species must be devised. One method for resolving these issues is plant multiplication through tissue culture. Plants that are genetically similar to their parent plants are produced by clonal propagation. Taking into account the value and viability of plant tissue culture technologies. Rhizome fragments are cultured in a manner that is limited to proliferation, differentiation, and regeneration, and this method has been employed for a wide range of therapeutic plant species (Nisa, 2018) [6].

2. Material and Method

The research work was carried out in the Department of Plant Biotechnology, College of Agricultural Biotechnology, Loni, and Affiliated by MPKV, Rahuri. The *Acorus calamus* plant was identified and purchased from Dhanwantari Nursery of MPKV, Rahuri. The fresh Rhizomes were collected, washed thoroughly under running tap water for 15min to remove any adhering dirt, followed by distilled water, excised rhizome explants were washed with savolon soap solution for 10 min, followed with distilled water for 3 min. Then the washed explants were taken into aseptic room and rinsed with double distilled water for 3-4 times. Explants were surface disinfected with 0.1%(w/v) Aqueous mercuric chloride (HgCl_2) solution for 1min and rinsed with double distilled water for 3 to 4 times. And then soaked in 70% ethanol for 30 seconds. The surface sterilized rhizomes were cultured on MS medium sterilized by autoclaving at 121 °C tempt, 15psi for 20 min. After inoculation all the cultures were grown under photo period of 16 hrs light (with white fluorescent tubes) at a temp of 25 ± 3 °C with different concentration and combinations of auxins for shoot formation. Data was recorded after 10 and

20 days of initial culture. The cultures having multiple shoots were excised and subcultured onto new medium. The MS medium 2.5 mg/l BAP + 1.0 mg/l IAA showed best growth in the Initiation stage therefore it was used again for the subculturing and multiplication stage. Similarly, for rooting, MS medium containing different concentrations of IBA and IAA from 1mg/l to 5 mg/l were first tested. Apart from single hormonal concentration, a combination of IBA and NAA was also tested for *in vitro* rooting of *Acorus calamus*.

3. Results and Discussion

Naturally grown, fresh rhizomes were cut into small pieces of about 1-3cm, were used as explants for shoot regeneration and multiplication. Regeneration of shoot from rhizome explant was observed at different hormone concentrations. The highest length of shoot was recorded at 2.5 mg/l BAP of around 8.125cm. and from the all other tested combinations of BAP+NAA and BAP+IAA, MS medium containing 2.5mg/l BAP and 1.0mg/l IAA showed highest average length of shoot of about 6.35cm

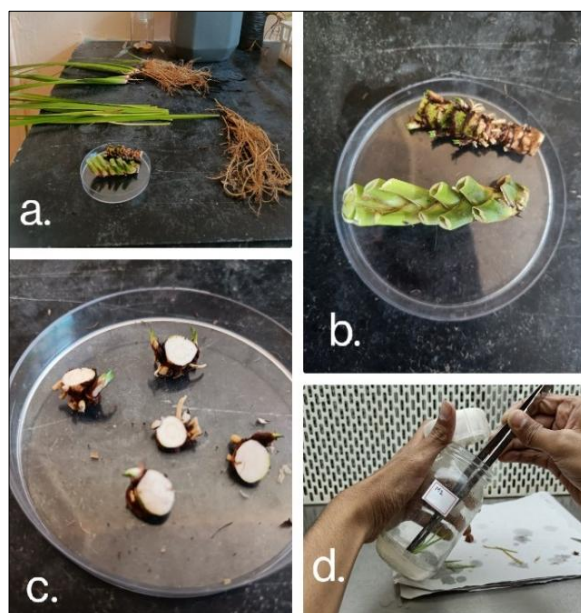


Fig 1: Washed *Acorus calamus* L. (a.), Excised rhizomes (b.), Rhizome segments Of 1-2cm (c.), Inoculation of sterilized rhizomes on MS medium (d.)

A. Shoot initiation result

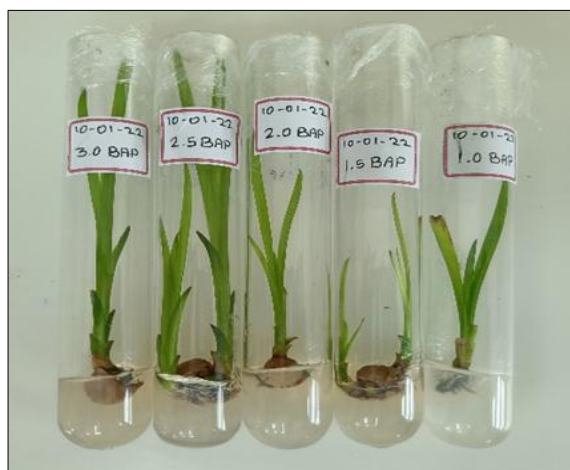


Fig 2: Shoot initiation on MS media containing 1mg/l to 3mg/l BAP

Table 1: Effect of 1.0 to 3.0mg/l BAP on *invitro* regeneration on *Acorus calamus* shoots

Bottle. No.	Media Combination	Average Length of Shoot
1	1.0 mg/l BAP	4.5
2	1.5 mg/l BAP	3.12
3	2.0 mg/l BAP	5.33
4	2.5 mg/l BAP	8.125
5	3.0 mg/l BAP	8

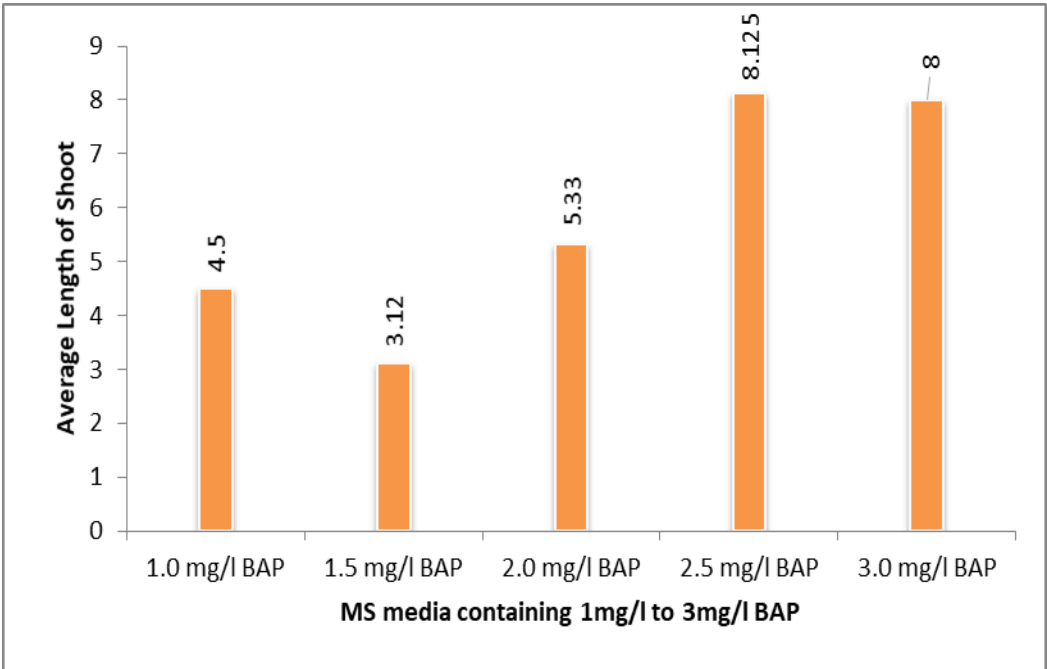


Fig 3: Average length of shoot on MS media containing 1.0 to 3.0mg/l BAP



Fig 4: Shoot initiation on MS media containing 2.5mg/l BAP with 0.5 to 2.5mg/l NAA

Table 2. Effect of various concentrations of BAP + NAA on *in vitro* regeneration on *Acorus calamus* shoots

Sr. No.	Media Code	Media Combination	Average Length of Shoot
1	A1	2.5 mg/l BAP + 0.5 mg/l NAA	2.5
2	A2	2.5 mg/l BAP + 1.0 mg/l NAA	1.5
3	A3	2.5 mg/l BAP + 1.5 mg/l NAA	6.2
4	A4	2.5 mg/l BAP + 2.0 mg/l NAA	3.8
5	A5	2.5 mg/l BAP + 2.5 mg/l NAA	2.75

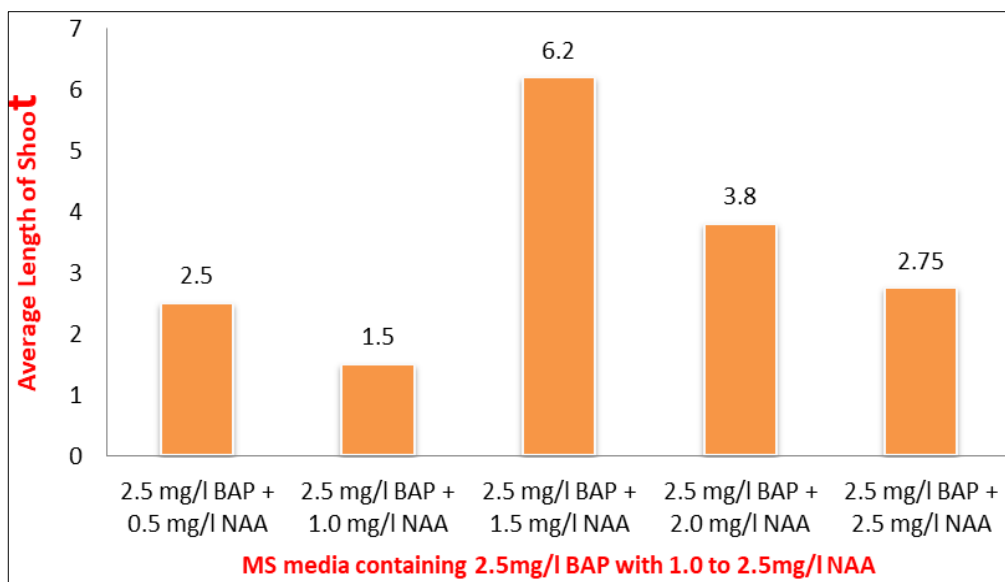


Fig 5: Average length of shoot on MS media containing 2.5mg/l BAP with 1.0 to 2.5mg/l NAA



Fig 6: Shoot initiation on MS media containing 2.5mg/l BAP with 0.5 to 2.5mg/l IAA

Table 3: Effect of various concentrations of BAP + IAA on *in vitro* regeneration on *Acorus calamus* shoots

Sr. No.	Media Code	Media Combination	Average Length of Shoot
1	B1	2.5 mg/l BAP + 0.5 mg/l IAA	6.31
2	B2	2.5 mg/l BAP + 1.0 mg/l IAA	1.5
3	B3	2.5 mg/l BAP + 1.5 mg/l IAA	6.35
4	B4	2.5 mg/l BAP + 2.0 mg/l IAA	3.8
5	B5	2.5 mg/l BAP + 2.5 mg/l IAA	3.16

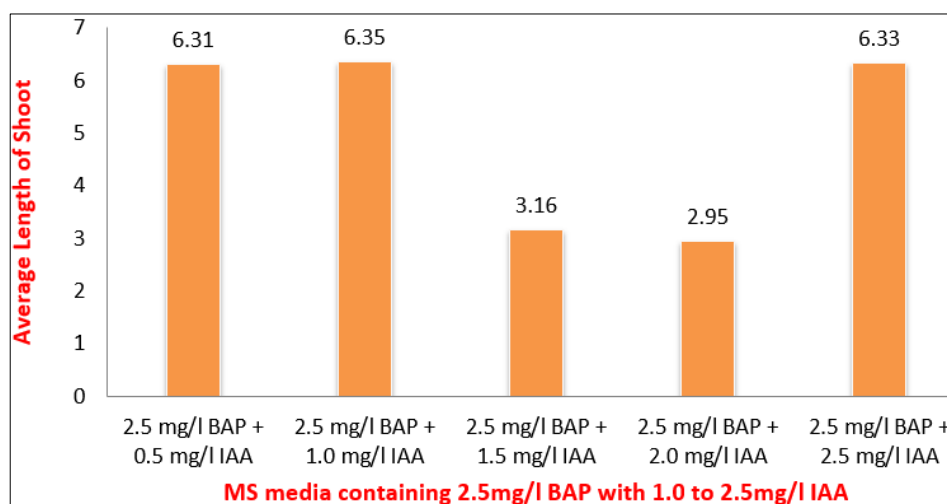


Fig 7: Average length of shoot on MS media containing 2.5mg/l BAP with 1.0 to 2.5mg/l IAA

Conclusion

It can be stated that all tested media for initiation could be used for initiation of Vekhand. Different growth hormones were used in tissue culture of *Acorus calamus* with variable results for comparative studies. When rhizome segment was cultured on MS medium having concentration of 2.5 mg/l BAP, maximum shoot formation was obtained. The MS Media containing 2.5 mg/l BAP was further tested along with various combinations of NAA and IAA. The best growth was obtained in the MS media containing 2.5 mg/l BAP along with 1 mg/l IAA.

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