Research Article

Effect of Different Growth Regulators on *In-vitro* Regeneration of Rhizome and Leaf Explants of *Acorus calamus* L.

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ABSTRACT

The genus Acorus is a perennial aquatic plant used as a medicinal and aromatic plant. *Acorus calamus* were cultured for in-vitro investigation of growth rate, shoot formation and root formation on the media containing different kinds and concentrations of plant growth regulators. In the first experiment, different concentrations of sucrose (2, 4, 6, 8, and 10% w/v) were examined to find the most effective concentration in induction of rhizome using the dual phase MS medium. The tested concentration of sucrose was able to induce microrhizomes in the works. The 6 % sucrose medium had the largest size (length 3.9 cm, diameter 0.47cm) and maximum fresh weight (0.72 g). The explants were cultured in various concentrations of cytokines like BAP and Kn and auxins like IAA, IBA, NAA, 2, 4-D individually as well as in diverse combinations. Highest numbers of shoots were observed with 3.0 mg/L NAA. Half strength MS medium having 1.0 mg/L IBA was found better with 50 percent of root formation after 15 days of vaccination with a upper limit number of roots (5) in the platelets were transferred to the greenhouse and survival rate was 95% after acclimatization.

Keywords: *Acorus calamus*, auxin, cytokinin, sucrose, rhizome

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INTRODUCTION

The flora world consists of numerous herbs that has wonderful medicinal importance which can be exploited by human for the betterment of healthcare and rid the society of many diseases. One such wonder plant is *Acorus calamus*, commonly known as Sweet Flag that has many medicinal values. It is a perennial herb which promotes circulation of blood to the brain, sharpens memory and enhances alertness, according to Ayurvedic medicine [1]. In traditional Chinese medicine, sweet flag is used to treat deafness, dizziness and epilepsy besides using it for vomiting, diarrhea, abdominal pain, and dysentery. All parts of this plant have a peculiar, agreeable fragrance, especially the roots which have a sweet fragrance and the leaves which produce a smell similar to lemon. In olden times, the plant was recommended by Taoists as having the power to best of immortality.

The rhizome is pale pink internally and contains an essential oil responsible for its medicinal value [2]. Major constituents of the essential oil are phenylpropanes, monoterpenes and thermolabile sesquiterpenoids. The rhizome produces glycoside, oxalic acid and volatile oil. The volatile oil is known as calamus oil that has been successfully employed in medicine since old time and at present it is used for the preparation of perfume, sacred oils, aromaticcordials, flavouring beer and liquor. Different herbal formulations for skin care, hair care, antacids, mosquito repellants and general tonics are available where this plant is a major ingredient [3]. The powdered rhizome is said to act as an antispasmodic, diaphoretic, expectorant and can cure tuberculosis as well as lung and heart cancer [4]. The rhizome is also used for treating diseases such as dyspepsia, flatulence, choleraic diarrhea in

children cough, fever, piles and asthma. This is also used as an insecticide, in the preparation of toilet powder and an antidote for several poisons. In the USA, the rootstock is often eaten raw for relief from indigestion.

MATERIALS AND METHODS

Explant preparation

The collected young rhizomes of *A. calamus* were soaked in water and brushed to remove the adhering soil. The leaf sheaths were carefully removed so that the young auxiliary buds were not injured. The rhizomes were washed in detergent solution for 10 min and rinsed with tap water. They were then surfaced sterilized with 0.1% (w/v) $HgCl_2$ solution having two drops of Tween-20 for 7 min and then rinsed with sterilized distilled water thrice. The rhizomes were trimmed and cut into pieces having a bud each.

Culture medium and condition

Murashinge and Skoog (MS) cmedium (1962) was used with B5 vitamins (Gamborg *et al.,* 1968), 3 % (w/v) sucrose, 0.8% (w/v) Difco bacto agar and supplemented with various concentrations of benzylaminopurine (BAP) and in combination with alpha-naphthalene acetic acid (NAA) for establishment of aseptic culture and multiple shoot induction. The pH of the medium was adjusted to 5.8 with 1 N NaOH or HCl and autoclaved at 121º C with 1.05 kg cm^{-2} pressure for 20 min. Single rhizome buds were inoculated per culture tube (32 x 200 mm) containing approximately 30 ml of the semi-solid agar medium overlaid with 5ml of the sterilized liquid fraction under sterile conditions. Except for agar, the liquid phase had the same organic, inorganic and growth regulator regime as that of the semi-solid phase for each treatment. The cultures were incubated at 25 ± 2 ºC under 28 $umol.m^{-2}\ s^{-1}$ illumination from cool white fluorescent tubes for 16/8 h light/dark photoperiod.

Multiple shoots induction

In-vitro grown shoots of *A. Calamus* approximately 2-3 cm long which were obtained from the established culture containing MS medium along with the 0.5 mg L-1 NAA and 2.0 mg L^{-1} BAP, were used as explants for induction of rhizomes. In the first experiment, a dual phase MS medium consisting of different concentrations of sucrose (2, 4, 6, 8, and 10 % w/v) was used for rhizome induction. Secondly, based on the result of the first experiment, the dual phase MS medium containing 6 % sucrose was supplemented with different concentrations of IBA (0.1, 0.5, 1.0, 2.0, and 4.0 mg. L^{-1}) and NAA (0.1, 0.5, 2.0, and 4.0 mg. L^{-1}) to test their effect on rhizome induction. There were fifteen replicates per treatment and the experiments were repeated twice.

Root induction

The rhizomes were harvested inside the laminar air flow chamber and the length, diameter and fresh weight of the rhizomes per plant were measured after six weeks of inoculation. Rhizomes of each plant with 7-8 buds were cut into segments having a single bud and washed with distilled water. In-vitro regenerated shoots (3.0 cm long) were excised aseptically and implanted on MS half and full strength, medium fortified with various concentrations (IBA - 0.5 mg/l to 2.0 mg/l) and NAA in an attempt to produce roots.

Acclimatization and hardening

Each plantlet was planted in sterilized sand in small PVC cups (120 ml volume). The cups were placed in a plastic tray with a layer of tap water (1cm) and covered with another plastic tray to maintain high humidity. Survival percentage was calculated over four weeks. The rooted plantlets were successfully acclimatized in pots containing sterilized soil and sand mixture (3:1) with 75% survival rate in the field conditions.

RESULTS AND DISCUSSION

Despite being a littoral plant, the explants sources were properly sterilized by treating in 5% fungicide for 15 min, 70 % alcohol followed by 0.1 % aqueous $HgCl_2$ solution for 7 min. In the first experiment, different concentrations of sucrose (2, 4, 6, 8, and 10% w/v) were tested to determine the most effective concentration in induction of rhizome using the dual phase MS medium. The different treatment showed a variable response in terms of size and fresh weight of the rhizomes (**Table1**). All the tested concentration of sucrose was able to induce micro-rhizomes in the plant. The 6 % sucrose medium had the largest size (length

3.9 cm, diameter 0.47cm) and maximum fresh weight (0.72 g). Size and fresh weight were gradually decreased with the further increase in sucrose concentration are given in figure 1 and 2. An advantage of the dual phase culture medium is that rhizomes could be induced even at a low sucrose concentration of 2 % after six weeks of inoculation while the same could not be observed on agar-gelled medium. It also has an advantage over liquid culture since it can anchor the explants in the agar gelled fraction and hence can be kept static without continuous shaking. Sucrose might act as an energy source and as an osmotic in inducing the rhizome formation [5]. Rhizome serves as sink where assimilates are uploaded, and in an in-vitro culture system assimilates provided as sucrose may have been transported to the stem for rhizome formation in gingers.

The work is similar to the study carried out by [6]. that developed a protocol for the regeneration of *Acorus calamus* L. In different growth regulators using naturally grown rhizome segments as the exploits. The explants were cultured on MS medium supplemented with various concentrations of cytokinins like BAP and Kn and auxins like IAA, IBA, NAA, 2, 4-D individually as well as in various combinations. Highest numbers of shoots were observed with the 3.0 mg L-1 NAA. Slightly better shoot multiplication was observed with BAP (2.0 mg L^{-1}) and NAA (0.5 mg L^{-1}) where 90% of explants showed proliferation. Similarly [7] achieved maximum shoot proliferation with 2.0 mg L^{-1} Kn and 0.05 mg L^{-1} NAA.

The excised in-vitro shoots failed to develop roots in half and full strength MS medium devoid of growth regulators. Half strength MS medium having 1.0 mg/l IBA was found better with 50 % of root formation after 15 days of inoculation with a maximum number of roots (5.0). Higher concentrations of IBA (1.5 mg/l and 2.0 mg/l) showed decreased percent of root formation. The plantlets showed 75% of survival rate in the field conditions.

Table 1: Regeneration of *A. calamus* from Rhizome Explants

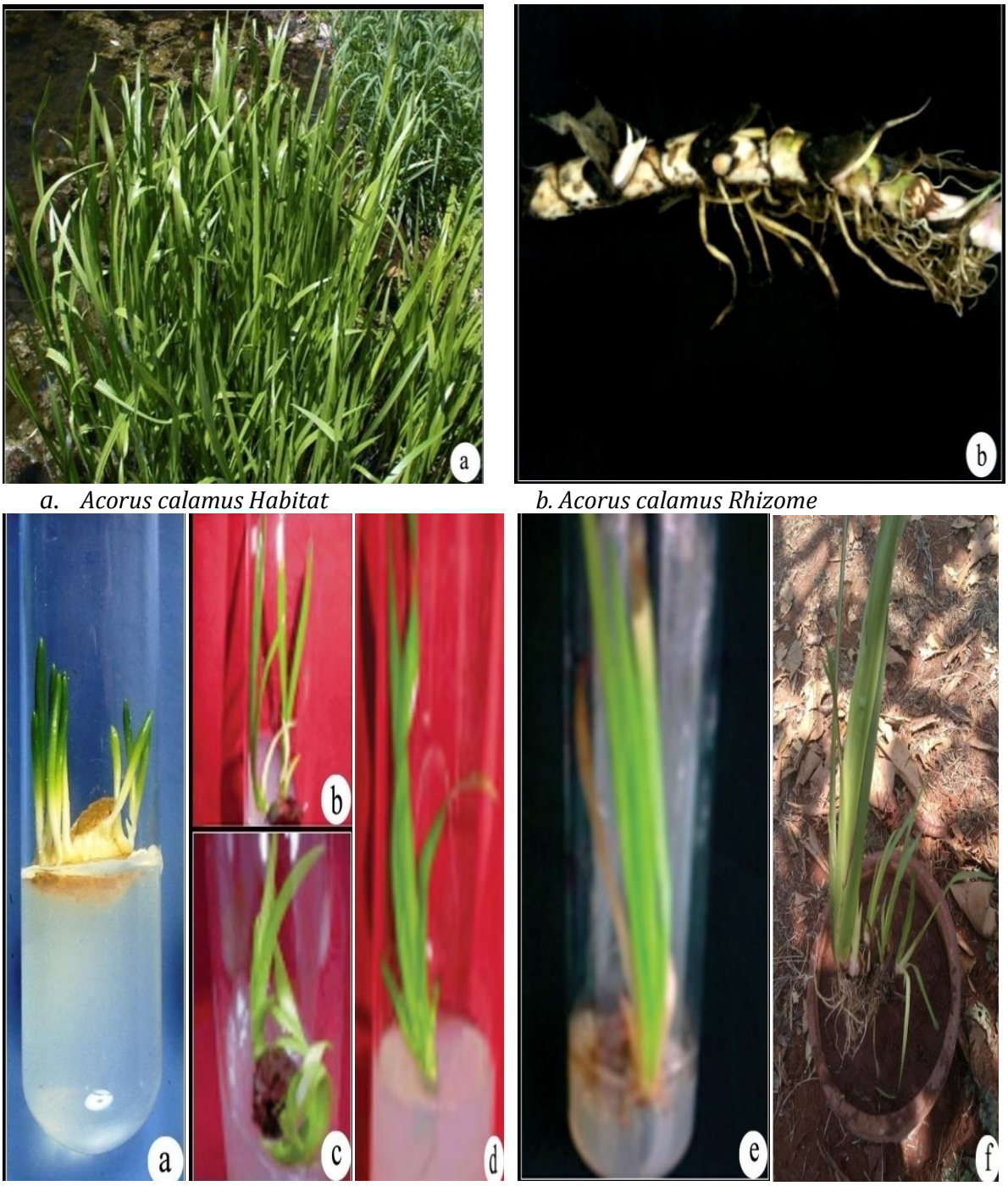
Growth regulators (mg L^{-1})	Shoot induction (cm)	Root induction (cm)
BAP+NAA		
1.0 0.5	1.2 ± 0.2^{f}	1.6 ± 0.2^{f}
1.0 1.0	1.8 ± 0.4^{e}	2.1 ± 0.4^{e}
BAP+IAA		
2.0 0.5	2.2 ± 0.2^{cd}	2.5 ± 0.5^{d}
2.0 1.0	2.4 ± 0.5^{c}	2.6 ± 0.4^{cd}
KIN+NAA		
3.0 0.5	2.6 ± 0.4^{bc}	2.7 ± 0.3^{c}
3.0 1.0	2.8 ± 0.2^{b}	3.0 ± 0.2^{b}
BAP+IAA		
3.0 1.0	3.0 ± 0.2^{ab}	3.2 ± 0.1^{ab}
3.5 1.0	3.2 ± 0.3^{a}	3.3 ± 0.2^{a}

Data represents mean values ± standard error (SE) of three replicates with 10 explants in each treatment and the experiment was repeated twice. Means with common letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

CONCLUSION

A. calamus values for its numerous medicinal properties. Some investigators have indicated that triploid *A. calamus* with less content of the ß - assertion may be the best raw material for the manufacture of herbal drugs or medicineIn the first experiment, different concentrations of sucrose (2, 4, 6, 8, and 10% w/v) were tested to determine the most effective concentration in induction of rhizome using the dual phase MS medium. The 6 % sucrose medium had the largest size (length 3.9 cm, diameter 0.47cm) and maximum

fresh weight (0.72 g). Size and fresh weight were gradually decreased with the further increase in sucrose concentration. An advantage of the dual phase culture medium is that rhizomes could be induced even at a low sucrose concentration of 2 % after six weeks of inoculation while the same could not be observed on agar-gelled medium. *A. calamus* The protocols for Micro induction and rooting described in this report will certainly help in large-scale clonal multiplication of the triploid accessions of this important medicinal plant for conservation and sustainable development.

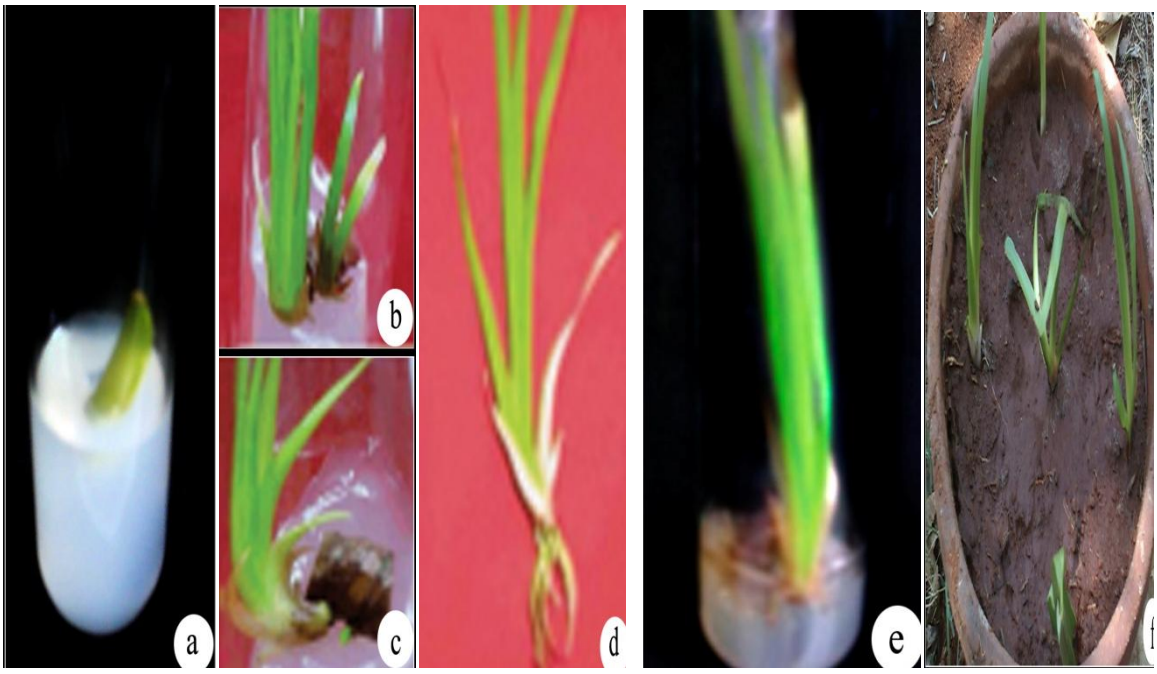


a. Acorus calamus Habitat *b. Acorus calamus Rhizome*

a. Rizome initiation; b. Invitro formation of shoots(7-10 days); c. Shoot multiplication after subculture(10-14 days); d. Shoot and root multiplication after subculture (15-30 days)
e. Regenerated plants; f. Hardened plant

Figure1: *In-vitro* Regeneration of Rhizome Explants of *Acorus calamus*

a. Acorus calamus habitat *b. Acorus calamus leaf explant*



a. Leaf initiation; b. Initiation of growth after 7 days; c. Sub cultured in vitro formation of shoot (10-15 days); d. Sub cultured in vitro formation of roots (15-30 days); e. Regenerated plants; f. Hardened plants

Figure 2: *In-vitro* Studies of Leaf Explants of *Acorus calamus*

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