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In Vitro Studies in Acorus calamus L

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ABSTRACT

Acorus calamus L (Acoraceae) is a medicinal plant of great commercial value and potential export crop. The practice of propagation of this plant is based on rhizome which is inadequate/limited for the planting material required for large scale cultivation. A rapid and efficient in vitro propagation protocol of Acorus calamus L. has been developed. The present investigation deals with the in vitro culture of Acorus calamus L (Sweet Flag) on Murashige and Skoogs (MS, 1962) medium fortified with different hormonal combinations and concentrations. Based upon the results it can be concluded that MS medium supplemented with 2,4 D (2 mgl^{-1}), 2,4 D (5 mgl^{-1}) + BAP (5 mgl^{-1}) and Kn (2 mgl^{-1}) results in leaf proliferation (Caulogenesis). Whereas medium augmented with BAP (2 mgl^{-1}), BAP (5 mgl^{-1}) + 2,4 D (5 mgl^{-1}), Kn (.5 mgl^{-1}) + 2,4 D (5 mgl^{-1}) + BAP (5 mgl^{-1}) + NAA (.5 mgL^{-1}), IBA (5 mgl^{-1}) + Kn (.5 mgl^{-1}) and Kn (1 mgl^{-1}) + BAP (5 mgl^{-1}) + NAA (.2 mgL^{-1}) proved best for shoot proliferation and rhizogenesis. The rooted plantlets were successfully acclimatized in pots containing sterilized soil and sand mixture (3:1) with 75% survival rate in the field conditions.

Key Words: Acorus calamus, micropropagation, medicinal, in vitro, explants

Abbreviations

BAP-6: Benzyl amino purine; **IAA**: Indole-3-acetic acid; **KN**: Kinetin-6 Furfurylamino purine; **NAA**: α – Naphthalene acetic acid; **MS** – Murashige and Skoog medium.

I.INTRODUCTION

Acorus calamus L. is used for micropropagation due to its potential medicinal uses. *Acorus calamus* L is commonly known as Sweet Flag (English), Bach (Hindi), Wadakaha (Sinhala) is a medicinal plant of great commercial value and potential export crop. *Acorus calamus* L. is a perennial herb, root system of adventitious type with thick branched rhizome, simple sword shape leaves. It grows to 1 metre in height. Venation parallel, petaloid with membranous sheathing base. It has yellow flower in summer Kumanan *et al.,* (2010). The leaves of wild or cultivated plant are blenched, crushed or chopped and applied to swellings, wounds, painful joints, tumours. A paste of the roots pounded finely together with a little ginger (*Zingiber officinalis*) is applied externally to cure bone aches. The rhizome is taken as a tonic and as an aphrodisiac, small amounts are thought to reduce stomach acidity, while large doses increase deficient acid production Mukherjee *et al.,* (2007). It is most effective antispasmodic, relieve spasm of the intestine. The plant remains a popular, herbal remedy Staden *et al.,* (2002) and Sharma *et al.,* (2014)). Rhizomes of *Acorus calamus* L. are beneficial in chronic, diarrhoea,

dyspepsia. It is a source of starchy food also used in perfumery Ahmed *et al.*, (2007). It is also added to bath preparations to alleviate nervous exhaustion Jayaweera (1982). The rhizomes of *Acorus calamus* L possess anti-spasmodic, carminative and anthelmintic properties and also used for treatment of epilepsy, mental ailments, chronic diarrhoea, dysentery, bronchial catarrh, intermittent fevers and tumors (Anonymous, 2000).

Due to its commercial importance and extensive use in medicines, there has been a growing demand for this plant. Conventionally, the plant can be propagated only by vegetative means. The plant rarely produces flowers or set seeds. The conventional propagation techniques of this plant are inadequate to provide the plant material required for large scale cultivation and to meet the growing need of pharmaceutical industry Hettiarchil *et al.*, (1997). Therefore, alternative means for the propagation of this plant species have to be developed

Plant propagation by means of tissue culture represents one of the approaches to overcome these problems. Clonal propagation gives rise to plants which are genetically identical to the parent plants. Considering the importance and feasibility of plant tissue culture technology. The process of culturing rhizome pieces is confined to proliferation, differentiation and regeneration which has been used for large number of medicinal plant species. Some work on micropropagation of *Acorus calamus* has been done by a few workers Hettiarchil *et al., (*1997) and Yadav *et al* (2012). Hence, in the present investigation *Acorus calamus* L. was undertaken with the objective for the development of efficient protocol for micropropagation and successful acclimatization.

II.MATERIAL AND METHODS

The present investigation was carried out at the AMU Aligarh (India). The young rhizomes of *Acorus calamus* L pieces measuring about 0.5 cm in length explants, were excised from the mature plant. After extensive washing the explants with running tap water for at least 30 minutes, they were immersed in 5% (v/v) Tween-20 for about 15 minutes. Subsequently, the explants were surface sterilized with 0.1% w/v $HgC1_2$ for 1 minute. After 4-5 extensive rinses with double distilled water, these explants were inoculated onto the culture medium.MS basal medium, Murashige and Skoog (1962), containing 3% (w/v) glucose, was used in all the experiments. Prior to the addition of 1% (w/v) agar and autoclaving at 15 lbs pressure and 121^0C for 15 minutes, the pH of the medium was adjusted to 5.8. MS medium was found to be the most suitable medium for inducing organogenesis and regeneration. Most preferred carbon source is sucrose, 3% sucrose is added in each combination. Before starting inoculation ultra violet (UV) tubes were switched on for 24 hours and inoculation was done in an aseptic chamber. After inoculation all the culture vials were kept under aseptically controlled environmental conditions at 25 ± 2^0 c temperature, 16/8 hour photoperiod at 35µEm-2 per flux for *in vitro* studies and 55-60 % relative humidity. Periodic observations were recorded regularly.

III.RESULTS AND DISCUSSION

Acorus calamus L. is an important medicinal plant propagated through rhizome only which limited the improvement. But through *in vitro* technique an attempt has been made to induce multiple shoot regeneration through rhizome culture. The present investigation deals with the *in vitro* culture of *Acorus calamus* L. on

Murashige and Skoogs 1962 (MS) medium fortified with different hormonal combinations and concentrations. Rhizome pieces measuring about .5 cm in length were cultured on MS medium containing different regimes of growth regulators. The rhizome explants cultured on Murashige and Skoog basal medium (controlled) remained as such for about 30 days after inoculation after that the explants died.

Explants cultured on MS medium supplemented with 2,4 D (2 mgl^{-1}) exhibited swelling after 6 days of inoculation followed by formation of 8 leaved primordia at three places within 12 days of inoculation. Fig.1A. Explants cultured on MS medium supplemented with BAP (2 mgl^{-1}) showed initiation of leaves and simultaneously rhizogenesis was also observed (Fig.1B) these results are in agreement with the findings of Rani *et al*., (2000), Jayaweera (1982) in the same plant and using medium fortified with IAA.

MS medium augmented with BAP (5 mgl^{-1}) + NAA (.5 mgl^{-1}) exhibited complete regeneration of plant Fig (1C and 1D) these results are in conformity with Jadhav *et al.,* (2017), Jayaweera (1982) in the same plant. Similarly explants cultured on MS medium fortified with 2,4 D (5 mgl^{-1}) + Kn (.5 mgl^{-1}) results in complete regeneration of plant (Fig. 2A, 2B). MS medium augmented with 2,4 D (5mgl^{-1}) + IBA (.2mgl^{-1}) exhibited caulogenesis, rhizogenesis and secretion of brown coloured secretion.(Fig. 2C) these results are in consonance with the findings of Jayaweera (1982) in the same plant however instead of brown secretion formation of callus was reported both in solid and liquid medium.

Explants cultured on MS medium fortified with Kn (1 mgl^{-1}) + BAP (5 mgl^{-1}) + NAA (.2 mgl^{-1}) showed rapid regeneration of roots along with single leaf primordial was noticed (Fig. 3A and 3B). These results are in agreement with the findings of Jayaweera (1982) in the same plant.

MS medium supplemented with 2,4 D (5 mgl^{-1}) + BAP (5 mgl^{-1}) showed formation of multiproliferation of leaves (Fig. 3C) these shoots subcultured on MS supplemented with IBA (1.5 mgl^{-1}) exhibited profuse rhizogenesis these results are in consonance with the findings of Shivani *et al*., (2012). However, medium augmented with half MS fortified with IBA (4.9 µM) obtained same results in the same plant Jadhav *et al.,* (2017). Rhizome pieces measuring 0.5 cm. in length were cultured on MS medium supplemented with BAP (2 mgl^{-1}) results in initiation of 10 leaf primordial followed by MS + 2,4 D (2 mgl^{-1}) results in 08 leaf primordial showed the best response for leaf proliferation and no rhizogenesis was observed. Similar findings have been reported in *Hypoxis rooperi* by Page *et al*., (1985) on MS + BAP (1 mgl^{-1}) and *Caladium sagipifolium* by Mujib *et al.,* (1996) on MS + 2,4 D (0.05-2.0 mgl^{-1}) or NAA (0.5-1.0 mgl^{-1}) with BAP. Proliferation and differentiation of leaves and roots was observed in the present investigation on MS + BAP (2 mgl^{-1}) alone or in combination with 2, 4 D, IBA, Kn and NAA have also been reported by Hetteriarchil *et al.,* (1997), Jayaweera (1982) and Kulkani *et al.,* (1999) Rani *et al.,* (2000) and Jadhav *et al*., (2017) in the same plant using MS medium supplemented with BAP or Kn (0.01 to 2.0 mgl^{-1}) along with IAA, IBA, NAA (0.02-1.0 mgl^{-1}) .

Based upon the results it can be concluded that MS medium supplemented with 2,4 D (2 mgl^{-1}), 2,4 D (5 mgl^{-1}) + BAP (5 mgl^{-1}) and Kn (2 mgl^{-1}) results in leaf proliferation. Whereas medium augmented with BAP (2 mgl^{-1}) , BAP (5 mgl^{-1}) + 2,4 D (5 mgl^{-1}), Kn (.5 mgl^{-1}) + 2,4 D (5 mgl^{-1}) + BAP (5 mgl^{-1}) + NAA (.5 mgL^{-1}), IBA (5

mgl^{-1}) + Kn (.5 mgl^{-1}) and Kn (1 mgl^{-1}) + BAP (5 mgl^{-1}) + NAA (.2 mgL^{-1}) proved best for leaf primordial initiation and rhizogenesis.

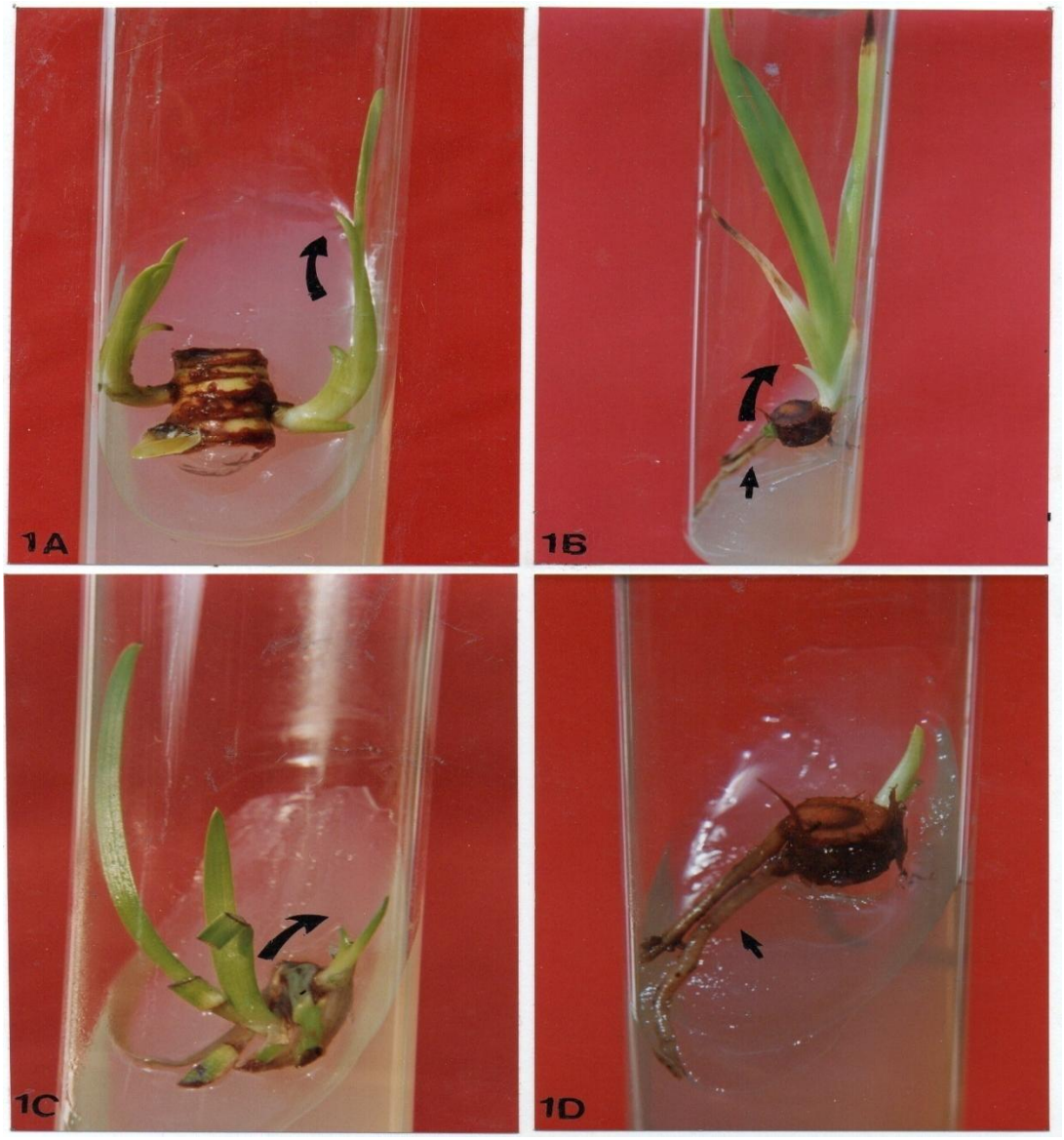

Fig. 1A 15 days old culture showing 10 leaf primordial at three places on MS + 2,4 D ($2mgl^{-1}$)

Fig.1B 66 days old culture showing 8 leaf primordial and rhizogenesis on MS + BAP ($2mgl^{-1}$)

Fig.1C 18 days old culture showing emergence of 04 leaf primordial from swollen region on MS + BAP ($5mgl^{-1}$) + NAA ($.5mgl^{-1}$)

Fig. 1D 9 days old culture showing initiation of leaf primordial and two well developed roots on MS + BAP ($5mgl^{-1}$) + NAA ($.5mgl^{-1}$)

Fig. 2A 22 days old culture showing profuse rooting and elongation of leaf primordial on MS + 2,4 D ($5mgl^{-1}$) + Kn ($.5mgl^{-1}$)

Fig. 2B Acclimatization in the earthen pot

Fig. 2C 25 day old culture exhibiting swelling, formation of leaf primordia, rhizogenesis and brown coloured secretion on MS + 2,4 D ($5mgl^{-1}$) + IBA ($.2mgl^{-1}$)

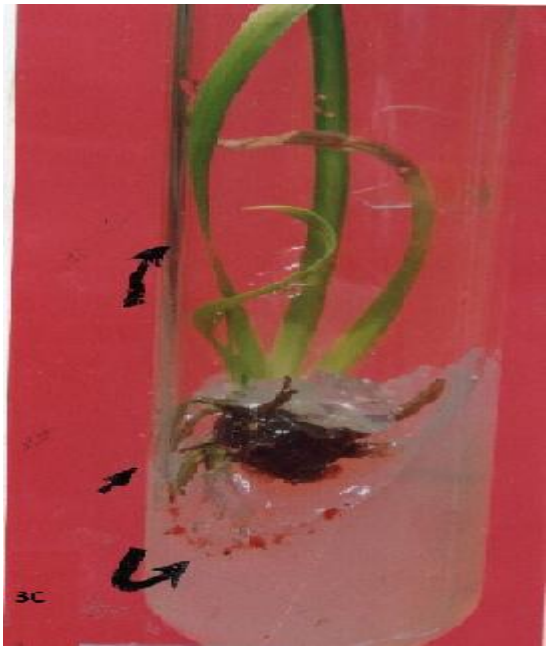

Fig. 3A Showing profuse brown coloured roots, along with elongation of leaf primordia on MS + Kn ($1mgl^{-1}$) + BAP ($1mgl^{-1}$) + NAA. ($.2mgl^{-1}$)

Fig. 3B Acclimatization in plastic pot

Fig. 3C Showing swelling, multiple proliferation of leaves, rhizogenesis and brown coloured secretion on MS + 2, 4 D ($5mgl^{-1}$) + BAP ($5mgl^{-1}$)

Table Response of rhizome in these combinations and concentrations

Medium	**Rhizogenesis**	**Leaf primordia**	**Remarks**
CONTROL	-	-	No response
MS+2,4 D (2 mgl^{-1})	-	8 in number	Swelling 4th D. A. I 08 leaf primordial at three places within 12 D.A.I. growth moderate.
MS+BAP (2 mgl^{-1})	02 roots	10 in number	Swelling 20th D. A. I multiple leaves (10) 31 D.A.I.rhizogenensis
MS+BAP(5mgl^{-1})+NAA (.5 mgl^{-1})	-	4 in number	Swelling 9th D. A. I four leaf primordial 18 D.A.I
MS+IBA(5mgl^{-1})+Kn (.5 mgl^{-1})	Profuse rooting	02 in number	Two leaf primordial 3 D. A. I, rhizogenesis 7 D.A. I
MS+Kn (2 mgl^{-1})	-	04 in number	Swelling 6 D.A.I first leaf primordial 12 D.A. I
MS+NAA(5mgl^{-1})+Kn (.5 mgl^{-1})	-	04 in number	Swelling 10th D.A.I first leaf primordial 17 D.A. I
MS+2,4 D (5mgl^{-1})+Kn (.5 mgl^{-1})	Profuse rooting	04 in number	Swelling 12th D.A.I first leaf primordia 15 D.A.I rhizogenesis 22 D.A.I
MS+Kn(1mgl^{-1})+BAP(5mgl^{-1})+NAA (.2mgl^{-1})	Profuse rooting	01 in number	Swelling 8th D.A.I rhizogenesis and leaf primordial on 11 D.A.I
MS+2,4D(5mgl^{-1})+BAP(5 mgl^{-1})	Profuse rooting	04 in number	Swelling 15th D.A.I first leaf primordial on 18 D.A.I rhizogenesis 27th D.A.I
MS+2,4D(5mgl^{-1})+IBA(.2 mgl^{-1})	Profuse rooting (5-6) in no.		Swelling 9th D.A.I first leaf primordia on 13th D.A.I few more leaves formed after 15th

			D.A.I
MS+BAP(5mgl^{-1})+NAA (.5 mgl^{-1})	-	04 in number	Four leaf primordia on 18 D.A.I

D.A.I-Days after inoculation

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