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CONSERVATION OF ENDANGERED MEDICINAL PLANT (*ACORUS CALAMUS*) THROUGH PLANT TISSUE CULTURE

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Abstract- *Acorus calamus* is a perennial herb and identified as an endangered species of medicinal plant. The genetic biodiversity of traditional medicinal plants is under a continuous threat due to over exploitation environment unfriendly harvesting and loss of growth habitat and unmonitored trade of medicinal plants. *Acorus calamus* is the botanical name of the plant more commonly known as calamus. Other common names of calamus include calamus root, flag root, muskrat root, sweet calomel, sweet flag, sweet sedge, and many other names. It has long been classified as belonging to the Araceae family, but more recent studies suggested that it should be placed in its own family. *In vitro* Micropropagation of *Acorus calamus* Plant was achieved using Rhizome Explant. The Explant was inoculated in M.S Media supplemented with different concentration of phytohormones(0.5-1mg/lit)IAA-BAP,(0.5-2mg/lit) IAA-BAP or without any growth Hormones. The frequency of Shoot Organogenesis is highest at 73% response rhizome treated with (0.5-2mg/lit) IAA-BAP and 30% response in treatment with (0.5-1mg/lit)IAA-BAP ,18% response in any pytohormone. The micro shoots rooted well in M.S medium supplemented with 4.0mg/l(IBA) Hardened regenrants(60%)acclimatized in soil.
Keywords:- Auxin, Cytokinin, Hardening, Organogenesis, Rhizome, Calamus oil, *Araceae*

Introduction

It is necessary to initiate systematic cultivation of medicinal plants in order to conserve biodiversity and protect endangered species. In the pharmaceutical industry, where the active medicinal principle cannot be synthesized economically, the product must be obtained from the cultivation of plants. Systematic conservation and large scale cultivation of the concerned medicinal plants are thus of great importance. Efforts are also required to suggest appropriate cropping patterns for the incorporation of these plants into the conventional agricultural and forestry cropping systems. Cultivation of this type of plants could only be promoted if there is a continuous demand for the raw materials.[1]. It is also necessary to develop genetically superior planting material for assured uniformity and desired quality and resort to organised cultivation to ensure the supply of raw material at growers end. Varieties of plants have been classified by their physical appearance and morphology. However, there may be large genetic differences between plants classified using this traditional method, and developing collections based on morphology alone may result in some genetic variations being lost. These variations are important, not only to conserve the species as fully as possible but also for retaining potentially useful properties in the plant.

Acorus calamus is an endangered plant species and of great medicinal importance, Sweet flag is a grass-like, rhizome forming, iris-like perennial that can grow to 2 meters high. It inhabits perpetually wet areas like the edges of streams and around ponds and lakes, in ditches and seeps. It often shares habitat with the common cat-tail. Plants have long creeping roots that spread just below the surface of the soil. Roots spread horizontally and can be up to almost 2 meters long, for old, well established specimens. Plants very rarely flower or set fruit, but when they do, the flowers are 3-8 cm long, cylindrical in shape, greenish brown and covered in a multitude of rounded spikes.[1] The fruits are small and berry-like, containing few seeds. Flowers from early to late summer. Calamus is associated with the muskrat in many native cultures as the rodent consumes copious quantities of the root. At present Plant tissue culture offers a valuable to overcome the problem regarding conventional propagation, and obtain disease free healthy plants, In this Examination Rhizome bud is used as an Explant and propagated in M.S Medium, this can ensure the production of healthy plants.

Materials and Methods

Selection of the explant depends on the species and type of the culture. The phenotypically superior plant should be selected as explant [2]. The plant should be disease free and healthy. Rhizome is selected as an Explant. Explant is to be washed 4-5 times with the double distilled water, then it should be kept in 5 % Extran solution for about 30mins after that it is to be washed 4-5 times with the double distilled water. Explant is kept in 2 % bevistein solution to make it free from fungal contamination for about 30mins, Explant is to be washed 4-5 times with the double distilled water and

then kept for UV Sterlization in the Laminar air Hood for 30 minutes after that 0.1 % HgCl2 should be added for surface sterilization for 5 minutes[5] . Explant was inoculated in M.S Media supplemented with different concentration of phytohormones(0.5-1mg/lit)IAA-BAP,(0.5-2mg/lit) IAA-BAP or without any growth Hormones.The frequency of Shoot Organogenesis is highest at 73% response rhizome treated with (0.5-2mg/lit) IAA-BAP and 30% response in treatment with (0.5-1mg/lit)IAA-BAP ,18% response in any any pytohormone. An excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots.[5]. A balance of both auxin and cytokinin will often produce an unorganised growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition. As cultures grow, pieces are typically sliced off and transferred to new media (subcultured) to allow for growth or to alter the morphology of the culture. The skill and experience of the tissue culturist are important in judging which pieces to culture and which to discard. As Carbon source 3%sucrose is added and 8% agar is added,pH is adjusted to 5.7-5.8 and the M.S medium contain various Macro, Micro Nutrients and Vitamins. [4]Tubes containing the explant should be kept in the culture lab where the temperature is maintained at 25 + 2 C. Explant has reached the appropriate size and length it should be subcultured in the appropriate medium, After attaining the desired size the plantlet should be transferred in the poly bags and kept in the Mist Chamber for 15 days after that transferred to the Green house and after acclimatization of the plantlet to the natural environment [5] .

Conclusion

To achieve the aim, experiments were conducted for micropropagation of *Acorus calamus* in M.S media with different concentration and different combination of plant growth regulators.

Acorus calamus: MS+NO PGR---- Moderate growth
MS+IAA(0.5mg/L) + BAP(1mg/L) – Rapid growth
MS+IAA(0.5mg/L) + BAP(2mg/L) – Extentsive growth

Sweet flag has a very long history of medicinal use in many herbal traditions. It is widely employed in modern herbal medicine as an aromatic stimulant and mild tonic. In Ayurveda it is highly valued as a rejuvenator for the brain and nervous system and as a remedy for digestive disorders.The root is anodyne, aphrodisiac, aromatic, carminative, diaphoretic, emmenagogue, expectorant, febrifuge, hallucinogenic, hypotensive, sedative, stimulant, stomachic, mildly tonic and vermifuge. It is used internally in the treatment of digestive complaints, bronchitis, sinusitis etc. It is said to have wonderfully tonic powers of stimulating and normalizing the appetite. In small doses it reduces stomach acidity whilst larger doses increase stomach secretions and it is, therefore, recommended in the treatment of anorexia nervosa. However if the dose is too large it will cause nausea and vomiting. Sweet flag is used externally to treat skin eruptions, rheumatic pains and neuralgia. An infusion of the root can bring about an abortion whilst chewing the root alleviates toothache. It is a folk remedy for arthritis, cancer, convulsions, diarrhoea, dyspepsia, epilepsy etc. Chewing the root is said to kill the taste for tobacco, because of these medicinal properties its conservation is must.

References

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Table 1- effect of hormone free media on rhizome

No. of test tube inoculated	No. of test tube responded	% Response	Morphogenic response					Remark
				7 day	14day	21 day	28 day	
17	3	18%	Average shoot Length	----	0.7 cm	2.5cm	5cm	Rapid growth observed ,contamination in 8 test tubes.
			Average no. of shoots	----	1	1	1	
			Average root number	----	----	----	2	
			Average root length	----	---	----	1.2 cm	
			Average number of leaves	----	4	4	4	
			callus.	----	----	----	-----	

Table 2- effect of iaa [0.5mg/l]+bap[1mg/l]on rhizome explant

No. of test tube inoculated	No. of test tube responded	% response	Morphogenic response					remark
				7 day	14da	21 day	28th Day	
20	6	30%	Average shoot Length	initiation	1 cm	4.3cm	6.5 cm	Rapid growth observed ,contamination in 4 test tubes.
			Average no. of shoots	----	2	2	2	
			Average root number	----	----	---- ---		
			Average root length	----	---	--- -	------	
			Average number of leaves	----	4	4	4	
			callus.	----	----	---- ---		

Table 3- effect iaa [0.5mg/l]+bap[2mg/l]on rhizome explant

No. of test tube inoculated	No. of test tube responded	Percen-tage response	Morphogenic response				Remark
				7 days	14 days	21 days	
15	11	73%	Average shoot Length	2cm	4.3cm	7.4cm	Rapid growth with no contaminat-lon
			Average no. of shoots	1	1	1	
			Average root number	---	----	------	
			Average root length	----	-----	---	
			Average number of leaves	4	4	4	
			Callus formation	----	----	----	

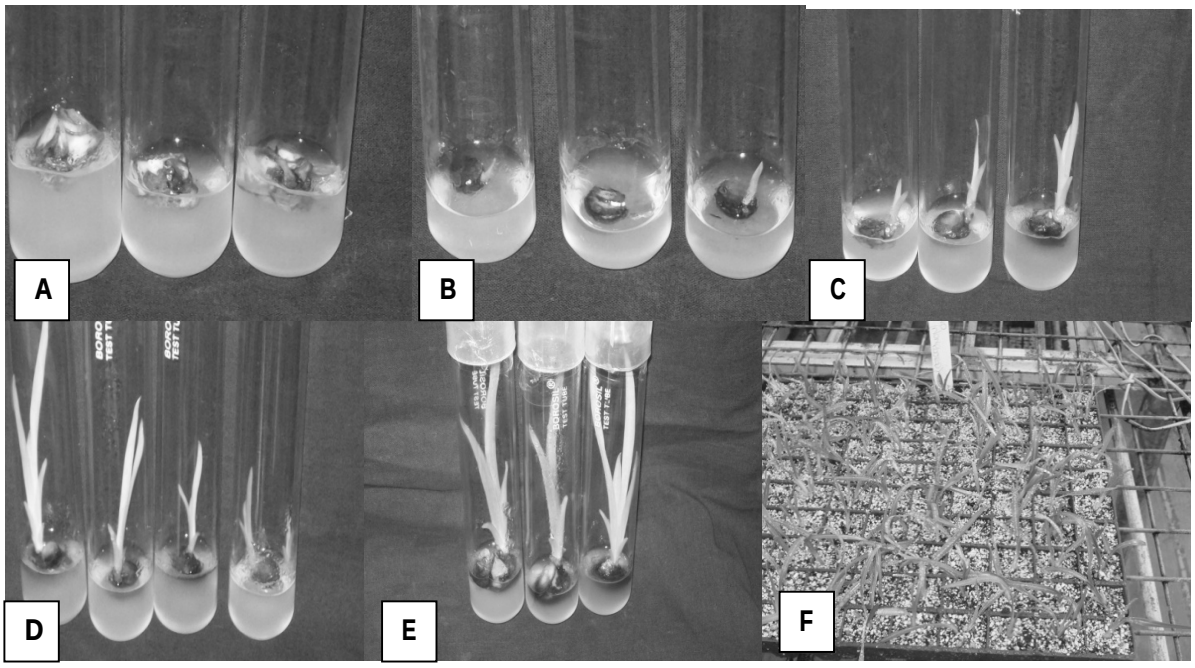

Fig. 1- A-Showing fresh culturing of Rhizome of *Acorus calamus*,B- Bursting after 14 days,Shoot initiataion after 21 days,C,D,E-Increase in length after 25 days,F – Hardening in Field condition