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Short Communication

In vitro Clonal Multiplication of *Acorus calamus* L

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***Acorus calamus* L (sweet flag) is a semi-aquatic perennial medicinal herb having a creeping and much branched aromatic rhizome. Rhizome buds cultured in MS basal medium supplemented with BAP (8.87μM) and NAA (5.37μM) at light intensity of 30μE m^{-2} s^{-1} and photoperiod of 12 h produced 8-10 multiple shoots by the eighth week of inoculation. Shoots could be rooted in MS basal medium without growth regulators. They were transplanted in sand and kept in shade for 4 to 5 weeks. 90-95% of the plants were established and were transferred to field after one month.**

Key words: micropropagation, sweet flag, *Acorus calamus*.

Acorus calamus (sweet flag) is found wild in many parts of India and is also cultivated in a few areas. The dried rhizome contains 1.5 - 3.5 % of volatile oil, the main constituent of which is asarone. The roots contain a glycoside, acorin. The dried rhizome is a common bazaar medicine, used as a tonic and carminative (1). The powdered rhizome is used for treatment of bronchitis, tumour, and kidney disorder in the Indian system of medicine and is a curative agent for carcinoma of lungs and heart (2). The oil of *A. calamus* is used in perfumery and also as an insecticide (3). The alcoholic extract inhibits growth of certain fungi (1). Calamus oil is highly priced, but it still remains an under utilized plant. The conventional method of propagation by vegetative buds produces only a single shoot and is time consuming for mass cultivation. Rapid propagation through micropropagation will be useful here for mass multiplication, for increasing the area under cultivation of this important medicinal plant. Micropropagation of sweet flag was reported by Harikrishnan *et al* (4). This paper describes a simple and efficient procedure for *in vitro* propagation of *A. calamus* from rhizome bud explants.

Rhizomes were taken from 2-3 years old *Acorus* plants maintained at IISR, Calicut. The rhizomes were first washed thoroughly in tap water; scales were removed and washed with liquid soap. It was then cut into small pieces and treated with 0.3% copper oxychloride for 20 min to minimise surface contaminants. Surface sterilization was carried out with mercuric chloride (0.1%) for 2-3 min under aseptic conditions and washed with sterile distilled water. Finally they were trimmed to 1cm bits, each with a bud and inoculated in culture media.

The basal medium used was MS (5) with 3% sucrose (Qualigens, India) and 0.7% agar (bacteriological grade, Hi media, India), autoclaved at 120°C and 1.5 kg cm^{-2} pressure for 20 min. The cultures were incubated at 25 ± 2°C under a 12-h photoperiod of 30μE m^{-2} s^{-1} light intensity provided by cool white fluorescent tubes. The basal medium was supplemented with combinations of BAP, 2-isopentyladenine (2ip), IBA and NAA. The pH of the medium was adjusted to 5.8 before autoclaving. The shoots developed were separated and transferred to rooting media after eight weeks. Shoots (7-8cm long) were transferred to MS medium with auxins IAA, IBA, NAA, 3% sucrose and 0.7% agar for rooting. Plantlets with well developed roots were transferred to cups after washing in running water to remove agar. The pots contained sand, soil and organic manure (cow dung) in the ratio of 3:1:1. The plants were kept under shade for four weeks before transplanting to the field.

For the production of multiple shoots, MS basal medium supplemented with cytokinins BAP, 2ip and auxins such as IBA and NAA were used (Table1). BAP was the best for eliciting shoot multiplication in *Acorus*. BAP alone induced 3-4 shoots in 40-50% of the cultures but, BAP in combination with NAA doubled the number of multiple shoots (Table 1). This combination induced 8-10 shoots by the eighth week of inoculation (Fig. 1A).

The superior effect of BAP on shoot bud induction and clonal multiplication was reported in pink ginger (6) and in *Curcuma* by Balachandran *et al* (7). The

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Table 1. Effect of growth regulators on shoot regeneration in *A. calamus*

BA	2ip	NAA	IBA	% response	No. of tillers* after 8 weeks
	(μM)				
2.22	0	0	0	40	3±0.82
4.44	0	0	0	50	4±0.87
8.87	0	0	0	50	4±1.2
0	0	2.69	0	30	2±1.11
0	0	5.37	0	40	3±0.83
0	0	10.74	0	50	4±0.97
4.44	0	2.69	0	60	4±1.21
4.44	0	5.37	0	60	5±1.23
8.87	0	5.37	0	80	8±1.45
8.87	0	10.74	0	70	5±0.88
2.22	0	0	2.46	40	3±0.92
2.22	0	0	4.90	40	3±0.97
4.44	0	0	2.46	50	4±1.1
4.44	0	0	4.90	60	5±1.23
8.87	0	0	4.90	60	5±0.99
0	4.92	0	0	50	3±0.73
0	9.84	0	0	50	4±1.32
4.44	4.92	5.37	0	60	3±0.92
8.87	9.84	5.37	0	60	4±0.72

Note - Data taken after two months of culture.

* Mean ± standard error of three experiments with 20 replicates each.

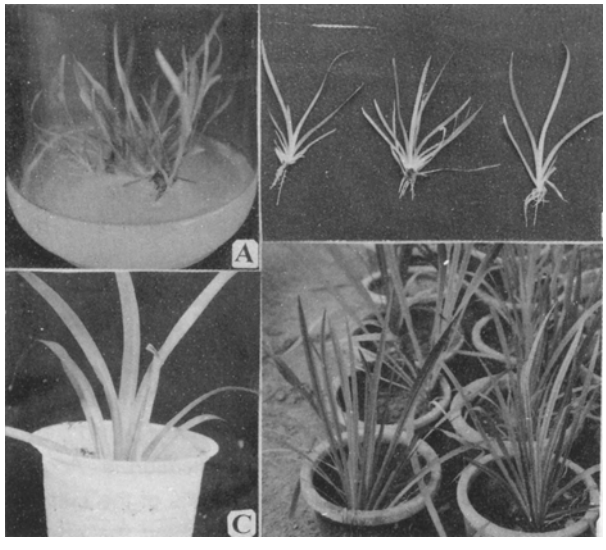

Fig. 1. *In vitro* propagation of *A. calamus* from rhizome bud explants. (**A**) Eight weeks old explant of *A.calamus* showing multiple shoot elongation, (**B**) Eight weeks old shoots of *A. calamus* transferred to rooting media. Left to right, MS, MS + IBA, MS + NAA, MS + IAA, (**C**) Hardened *A. calamus* plant, and (**D**) *A.calamus* tissue cultured plants in the shed.

Table 2. Effect of growth regulators on rooting mutiple shoots of A. *calamus* after one month of culture

IBA	IAA	NAA	No. of roots*	Length of roots* (cm)
	(μM)			
4.90	0	0	5±1.56	4±2.57
9.80	0	0	7±2.10	6±1.43
0	5.71	0	4±1.83	5±1.32
0	11.42	0	6±0.93	7±1.01
0	0	5.37	5±1.98	1±0.83
0	0	10.74	3±1.86	0.5±0.25
0	0	0	8±1.95	7±2.87

* Mean ± standard error of three experiments with 20 replicates each.

combination of BAP and NAA induced shoot multiplication in *Acorus* (4) and in pineapple (8). BAP along with NAA also induced multiple shoots in *Alpinia* (9).

The shoots developed were transferred to rooting media containing NAA, IBA and IAA (Table 2). MS basal medium without growth regulators produced 6-8 roots of 5-7 cm length after four weeks of culture (Fig.1 B). Rhizogenesis in *Acorus* was inhibited in the presence of high concentrations of NAA and produced short stumpy roots. Similar observation was also reported by Harikrishnan *et al* (4).

The shoots developed *in vitro* without roots when given a pulse treatment with IBA (0.5 mg l^{-1}), developed healthy fibrous root system. The rooted plantlets when transferred to pots registered 80 to 90 % survival and were transferred to the field after one month. No humid chamber phase was required for hardening tissue cultured plants of *Acorus*.

Large-scale multiplication of this precious medicinal plant can be achieved by this protocol. Earlier workers could get a multiplication rate of 5 shoots per bud only. The present protocol produced 8-10 harvestable shoots every eighth week without any decline in multiplication rate.

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