



Original Research Article

Efficient protocol for micropropagation of *Calamus huegilianus* – an endangered taxon

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ABSTRACT

Keywords

Rattans,
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Calamus huegilianus is a rare and endangered taxon of the Western ghats, India. Shoot tips of three month old seedlings were inoculated onto MS and L2 media supplemented with various growth regulators to record their morphogenetic potential. Both direct and indirect organogenesis was recorded depending on the type and concentration of hormones supplemented to the media. L2+BAP (2mg/l) and L2+NAA (2mg/l) + BAP (4mg/l) were proved to be the best combination for direct and indirect regeneration. Thus obtained shoots were rooted on L2 supplemented with either IAA or NAA. Acclimatized regenerates in vermiculate and perlite were transferred to field. Nearly 40% of survival was recorded.

Introduction

The members of the genus *Calamus* of Areaceae, commonly known as canes are categorised under Rattans along with other 12 genera. Although rattans are classified as minor forest products, their commercial and social importance is second only to timber in South East Asian countries. Habitat destruction, over exploitation and unscientific harvesting methods for the past few decades resulted in a drastic depletion of *Calamus* resources in India (Lakshmana, 1993).

Considering the rate at which tropical forests, the natural habitats of rattans are being destroyed, effective measures are to be taken to conserve and propagate the

threatened species. Supply of quality seeds of desired species is difficult because of inaccessibility of plants, insufficient quantity and problems of storage. Further, extraction of canes before flowering drastically affects the seed source.

Calamus huegilianus Mart. is an endangered taxon with limited population in the Nilgiris and Subramanya range of Western ghats (Renuka and Anto, 1998). It is one of the good quality canes among rattans but not available in sufficient quantities (Renuka, 2000). Hence, the present study is an attempt to develop an effective *in vitro* protocol to micropropagate this taxon, a step forward towards its conservation.

Material and Methods

Seeds and seedlings were collected from Subramanya range of Western ghats. Seedlings were maintained in the department poly house. Shoot tips (0.5 mm); nodal segments (1 cms) and leaf segments (1 cm²) were excised from 3 months old seedlings. They were washed thoroughly in running water with teepol for 30 min.

The explants were then surface sterilized with bevestin (fungicide) for 30 min and mercuric chloloride for 1 min. They were washed with sterile distilled water after each treatment to remove the sterilizants. Murashige and Skoog (MS, 1962) and Philips and Collins (L2, 1979) media were used in the present study. Auxins – 2,4-D, IAA, IBA, NAA and cytokinins – Kin, BAP and 2-ip were supplemented to nutrient media at various concentrations either alone or in combinations. Sucrose (3%) and Bacteriological grade Agar agar (0.8%) were used as carbon source and gelling agent respectively. The p^H of the media was maintained at 5.6 and autoclaved for 15 min at 109 kpa. The cultures were maintained under white fluorescent tubes with 16:8 h light and dark regime at the intensity of 25 μmol m⁻²s⁻¹.

Data analysis

The recorded data were represented as ± standard error based on five replications. One way ANOVA was used for analysis of data, significant 'F' ratios between groups means were further subjected to DMRT using SPSS version 15. Probability values <0.05 were considered as significant.

Results and Discussion

Calamus huegilianus is a clustering high climbing moderate sized cane. Shoot apices, root tips and young leaf segments were excised from healthy seedlings and

inoculated on MS and L2 media supplemented with growth hormones either alone or in combinations. Shoot apices were responded positively to the culture conditions than other explants. None of the explants responded on hormone free media. However, the morphogenetic response of explants on the media supplemented with various growth regulators varies depending on the type, concentration and combinations of the growth hormones (Table 1).

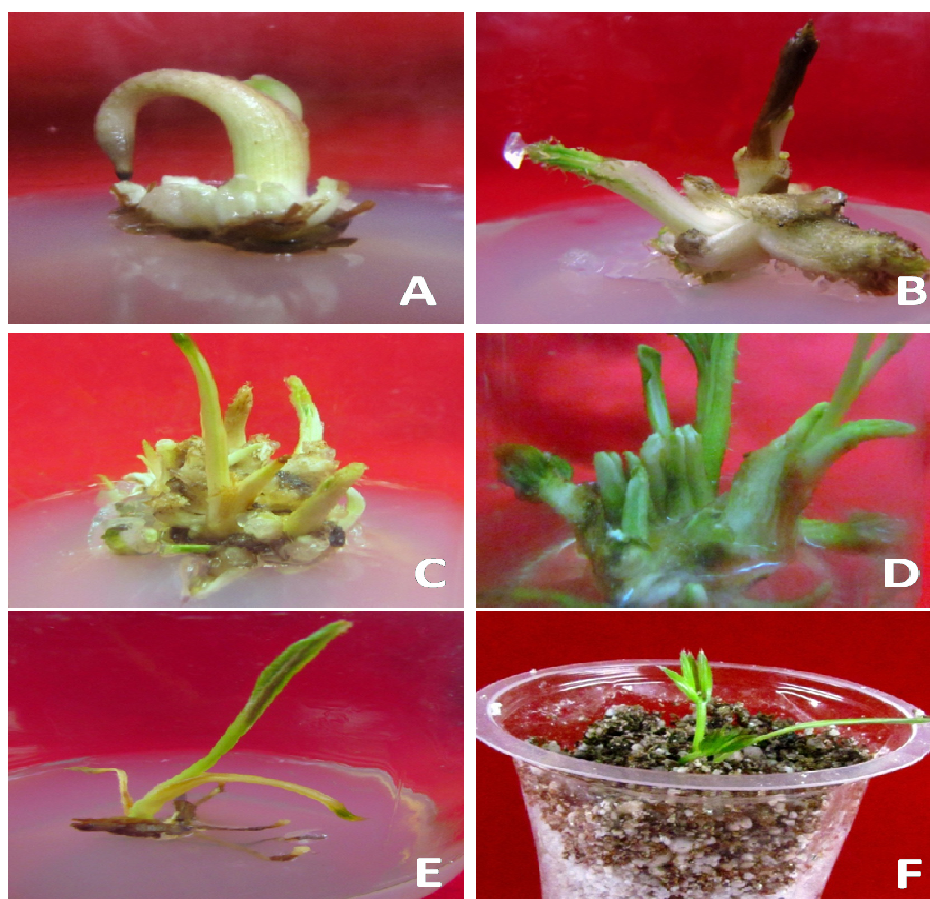
A critical balance between the endogenous and exogenously supplied growth regulators is needed to promote the cultured tissues to develop either into a shoot / or root and somatic embryos (Skoog and Miller, 1957). Presence of 2,4-D (2 mg/l) and NAA (5 mg/l) in the medium promoted the proliferation of shoots from the shoot apical region (Fig.1A). In none of the previous reports on the tissue culture in rattans, 2,4-D has promoted the induction of multiple shoots. Instead, profuse callus induction was reported from explants of various rattans in presence of 2,4-D in the medium (Yusoff, 1989; Hemanth Kumar et al., 2013; Krishna Kumar et al., 2012a; 2012b).

Induction of multiple shoots from the shoot apices in presence of 2, 4-D and NAA may be due to the change in the levels of endogenous cytokinins as a part of auxin – induced organogenesis (Pernisova et al., 2007). Reinhardt et al., (2010) were of the opinion that auxin is required for and sufficient to induce organogenesis both in the vegetative tomato meristem and in *Arabidolopsis* inflorescence meristem. Among the cytokinins supplemented to the media, BAP at 2 mg/l had promoted 3 to 4 shoots from the explants without the formation of callus(Fig.1B).Direct organogenesis was reported from embryo cultures of *C.andamanicus* on the medium supplemented with Kinetin (Valsala and Muralidharan, 1999).

Table.1 Effect of various growth regulators on multiple shoots regeneration in the shoot tip cultures of *Calamus heugelianus*

S. No.	Media+ Plant Growth Regulators	Shoot Number \pm SD
1	L2+2,4-D(2 mg l^{-1})	2.6 \pm 0.52
2	L2+NAA(4 mg l^{-1})	2.7 \pm 0.82
3	L2+BAP(2 mg l^{-1})	1.8 \pm 0.79
4	L2+2ip(4 mg l^{-1})	11.5 \pm 2.12
5	L2+2,4-D(0.5 mg l^{-1})+BAP(2 mg l^{-1})	4.7 \pm 0.82
6	L2+2,4-D(2 mg l^{-1})+BAP(4 mg l^{-1})	3 \pm 0.82
7	L2+NAA(0.5 mg l^{-1})+BAP(2 mg l^{-1})	9 \pm 1.76
8	L2+NAA(2 mg l^{-1})+BAP(3 mg l^{-1})	2.7 \pm 0.67
9	L2+IAA(2 mg l^{-1})+BAP(4 mg l^{-1})	5.1 \pm 1.73
10	L2+NAA(2 mg l^{-1})+BAP(4 mg l^{-1})	11.6 \pm 0.84

Figure.1 Multiple shoot induction from the shoot tip cultures A: Direct multiple shoot regeneration on L2+2, 4-D (2mg/l); B: Direct multiple shoot regeneration on L2+BAP (2mg/l); C: Callus induction and regeneration of multiple shoots from the callus on L2+2-ip (4mg/l); D: Multiple shoot regeneration from the callus cultures on L2+ NAA (2mg/l) + BAP (4mg/l); E: Induction of roots from the basal part of regenerated shoot L2+IAA (4mg/l); F: Acclimatization in vermiculite and perlite (1:1) mixture



Yusoff (1989) was able to induce multiple shoots from the color region of the *in vitro* raised seedlings of *C. manan* on MS fortified with BAP/Kin.

Cytokinins are known to induce cell division and development of meristmatic centres leading to the formation of organs (Peeters et al., 1997). In the present study, explants started callusing after four weeks of culture on 2-ip (4 mg/l) supplemented medium. Green shoot buds were initiated from the cremish nodulated callus after 6 weeks of culture. Maximum number of 15 shoots per culture was recorded (Fig.1C). In *C. nagabettai*, 2-ip at lower concentration (1 mg/l) had promoted the formation of maximum member of shoot buds (23) from the shoot tip callus cultures (Tejavathi et al., 2013). Kin and BAP either alone or in combination had supported the formation of shoot buds from the auxin derived callus in *Calamus travancoricus* and *C. nagabettai* (Kumar et al., 2012a, 2012b).

Synergistic effects of auxin and cytokinin on the morphogenetic potential of the explants was studied by fortifying MS / L2 medium with combination of hormones. The responses of the cultures varied depending on the type and concentration of auxin – cytokinin combinations. Low concentrations of auxins combined with high concentrations of cytokinins have been used for direct induction and elongation of multiple shoots in various systems (Khan et al., 1999). As was reported earlier in *C. nagabettai* (Tejavathi et al., 2013), Kin along with auxins promoted only the growth of the shoot apex into a single shoot even in the present study. However, BAP with 2.4-D / NAA had induced the formation of cremish nodulated callus after four weeks of culture. 2.4-D and NAA are more effective in combination with BAP in inducing multiple shoots from the callus cultures. Maximum number of 15 shoots per culture was

recorded on L2+NAA (2mg/l) + BAP (4mg/l) (Fig.1D).

Rooting and acclimatization of regenerated plantlets

Thus obtained shoots from direct and indirect organogenesis were transferred to media containing IAA/IBA/NAA at various concentrations for root induction. IBA is the most favored auxin for induction of roots in many species (Arya et al., 1999). However, in the present study, IBA was not efficient in inducing roots from the shoots. NAA at 2 mg/l and IAA at 4 mg/l had promoted the formation of roots from the basal parts of the shoots(1E). In *C.flagellum* NAA had promoted maximal number of roots than IAA and IBA (Kundu and Sett, 1999). The rooted plantlets were removed carefully from the medium and washed thoroughly to remove the traces of media and transferred to plastic cups containing vermiculate and perlite (Fig.1F). After 30 days of hardening in vermiculate and perlite, plantlets were transferred to pots containing soil:sand:manure at 1:1:1 ratio before planting them in field. Nearly 40% of survival was recorded.

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