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EFFECT OF PLANT GROWTH REGULATORS ON CALLUS GROWTH OF *Cola nitida* (Malvales: Sterculiaceae)

O. O. Obembe*, A. C. Adebona and E. B. Esan**

Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria

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ABSTRACT: The effects of plant growth regulators, naphthl-1-acetic acid (NAA) and 6-benzyl-1-1-aminopurine (BAP) on callus growth of leaf discs and nodal sections from *Cola nitida* (Vent.) Schott and Endlicher were investigated. The auxin concentrations used for the leaf explants were 1.0, 3.0, 5.0 and 7.0 mg/l NAA, while for the nodal explants, the concentrations employed were 0.5, 1.0, 1.5 and 2.0 mg/l NAA. Each was also separately combined with 2.3 mg/l BAP. the best callus growth was obtained on half-strength Murashige-Skoog (MS) media supplemented with 1.0 mg/l NAA and 2.3 mg/l BAP. The greatest callus growth of nodal explants was also observed with media having 1.0 mg/l NAA and 2.3 mg/l BAP. Calluses from the node cutting explants showed tendency for cell differentiation. In general, nodal explants responded to treatments better than leaf explants.

Key Words: Tissue culture; Stimulant crops; *Cola nitida*; Callus growth; Plant growth; Regulators.

INTRODUCTION

Cola nitida (Vent.) Scott and Endlicher (Kola) is a stimulant plantation crop of the tropics and subtropics (Eijnatten, 1973), Kola nuts have high social, ceremonial and industrial value. Owing to the stimulatory effects of the nuts, Kola could be grouped under non-alcoholic caffeine beverage crops like coffee (*Coffea* spp.), tae (*Camellia* spp.) and cocoa (*Theobroma caocao* L.).

However, its drawback has been its low seed germination resulting in poor development of fruit/seed/ cotyledon, arising from sterility/incompatibility (see Adebona, 1992 for review). Several *in vivo* studies aimed at overcoming some of these problems have not yielded positive results. Thus, it was of interest to explore tissue culture studies as a means of overcoming the problem of seed germination in Kola.

Literature reports on achievements in plant tissue culture of some economically important tropical plantation crops was reviewed by Esan (1992). Somatic embrypgenesis of cocoa was achieved from embryo axes of mature seeds and cotyledons of immature embryo (Esan, 1977).. Successful germination of the somatic embryos and rearing of field grown plant was achieved after about two decades (Sondahl, 1992; Lopez-Baez *et al.*, 1994). Tissue culture research on tea had been able to achieve regeneration of structures which include plantlets, pseudobulbis, shoots, roots, cotyledons and somatic embryos (Saha and Bhattacharya, 1988; Yan *et al.*, 1988; Kato, 1989). The induction of somatic embryos from leaf cultures of *Coffea* spp. had also been reported (Pierson *et al.*, 1983; Hatanaka *et al.*, 1991).

A review of the literature showed that information on Kola tissue is scanty. Thus, the objective of the work reported here was to investigate the appropriate media requirements for callus growth on Kola tissues and organs in order to develop an appropriate protocol for its *in vitro* regeneration.

*Present Address: Department of Biological Sciences, University of Agriculture, Abeokuta, Nigeria.
**Present Address: Plant Breeding Department, Cocoa Research Institute of Nigeria, Ibadan, Nigeria.

MATERIALS AND METHODS

Callus induction was established on leaf discs and nodal sections cultured on modified MS medium (Obembe, unpublished data). The induced calluses were transferred to growth inducing medium (GIM) after six weeks of culture.

Leaf callus growth media

Full-strength and half-strength modified MS media were tested as basal media for leaf callus growth. The concentration of NAA employed for leaf callus induction (0, 0.0, 1.0, 3.0 and 5.0 mg/l) were replaced by 0, 1.0, 3.0, 5.0 and 7 0 mg/l NAA respectively. Each concentration was separately combined with 2.3 mg/l BAP for both the full- and half-strength modified MS media. Replenishment of the media were made after the eighth week.

Nodal callus growth media

The callus inducing medium (CIM) concentrations (0.1, 0.5, 1.0 and 1.5 mg.l NAA) employed for nodal callus induction were replaced by 0.5, 1.0, 1.5 and 2.0 mg/l NAA respectively. BAP concentration was maintained at 2.3 mg/l as for the CIM.

Nodal callus differentiating medium

Callus transfer was made after seven weeks, from the CIM to callus differentiating medium. Tha basal medium, modified MS medium was gelled with 0.5% agar. 100 mg/l asparagine was incorporated. The cytoleinin level as maintained at 2.3 mg/l BAP while the auxin level (1.0 mg/l NAA) was replaced by the initial concentration in the CIM, 0.5 mg/l NAA.

RESULTS AND DISCUSSION

The investigation conducted with full-strength modified MS medium supplemented with NAA gave considerable callus growth in all treatments (Fig. 1, a-d). Increase in the diameter of the explants cultured on the control medium was observed after a week of transfer (Fig. 1a). Rolling up of explants as well as pronounced browning of the explants was observed on cultures of 5.0 mg/l (Fig. 1d) and 7.0 mg/l (data not shown). In general, callus size increase was observed with corresponding increase in browning (necrotic effects) in all cultures. However, only the callus on 1.0 mg/l NAA survived till the eighth week. This suggests that *C. nitida* leaf callus can be best maintained with 1.0 mg/l NAA. Cunha *et al.* (1994) also reported that rice callus development and growth was favoured by NAA (0.5 – 5.0 mg/l). However, Odewale (1983) reported that the best medium for oil palm anther callus growth was the one without any growth regulators.

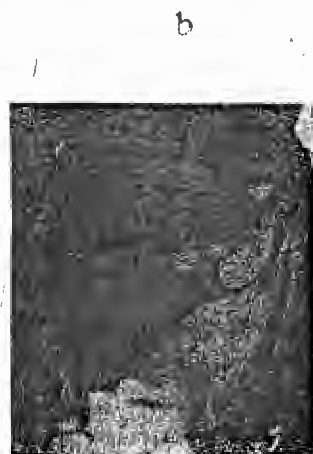
Considerable size increase of the calluses was observed in all the cultures on half-strength MS medium barely after the second week (Fig. 1, (e-h). In spite of the browning effects, size increase continued in all the cultures till the eighth week, except for the control. However, only calluses on 1.0 mg/l and 3.0 mg/l NAA survived till the tenth week after replenishment of the CIM (Fig. 1, k&l). With the survival time of four and half months, it is suggested that callus growth is better maintained on half-strength medium than on full-strength. The use of half-strength medium was found satisfactory for *in vitro* studies of cocoa which shares the same family with Kola (Litz, 1986; Novak *et al.*, 1986; Adu-Ampomah *et al.*, 1988). Park *et al.* (1993) also reported that callus formation and growth from immature haploid wheat embryo were greatest on half-strength MS medium supplemented with 1 mg/ml 2,4-D.

The results obtained from investigation on callus growth derived from nodal sections in this work are more encouraging than those obtained for leat cultures. Small protuberances were observed at the top of the calluses maintained on 1.0 mg/l and 5.0 mg/l NAA after the third week of transfer (Fig. 1, j & l). Considerable callus growth was recorded for callus on 1.0 mg/l NAA till the seventh week. The small protuberances observed on the calluses were also reported by Esan (1973) and had been described as certain globular or spherical morphogenic manifestations which can actually develop into embryoids or ordinary shoots.

Observations made after seven weeks of subculture of the only growing callus with protuberances (1.0 mg/l NAA) on differentiating medium showed that the creamy white colour of the callus was observed to be changing to green. No further differentiation was observed at the time of this report. Yadav *et al.* (1995), however, reported shoot regeneration from leaf midrib-derived tobacco callus cultures on LS medium supplemented with 2.0 mg IAA, 20 mg kinetin, 160 mg adenine sulphate and 340 mg NaH_2PO_4/litre.

a

b



c

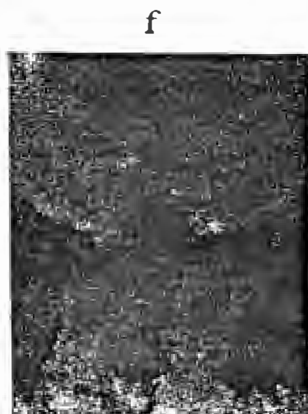
d

a – d: *C. nitida* leaf explants' calluses maintained on full-strength modified MS supplemented with 0 – 5.0 mg/l NAA and 2.3 mg/l BAP.

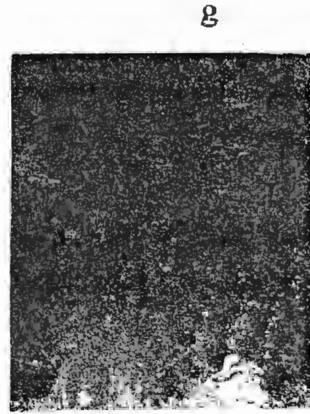
(a) control, displaying increase in size of the explants and callus growth.
(b) considerable callus growth in 1.0 mg/l.
(c) 3.0 mg/l showing callus growth as well as necrosis.
(d) 5.0 mg/l displaying pronounced necrosis and rolling up of explants.

e

f



g

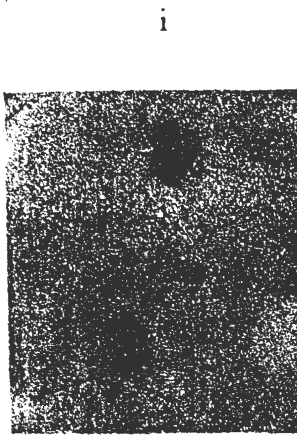


h

e – h: Explants' calluses maintained on half-strength modified MS, supplemented with 0 – 5.0 mg/l NAA and 2.3 mg/l BAP.

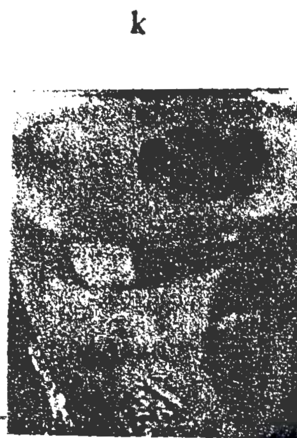
(e) control, showing growing calluses.
(f) 1.0 mg/l.
(g) 3.0 mg/l
(h) 5.0 mg.l showing best callus growth as at two weeks in growth media

i

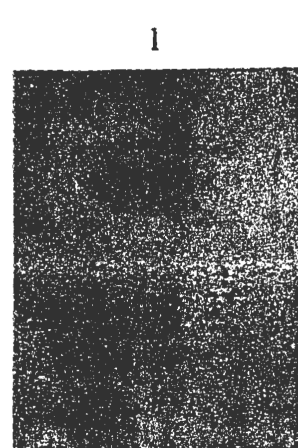


j

k



l



Nodal Nodal explants' calluses maintained on full-strength modifiec MS supplemented with 0.5 mg/l d 1.0 mg/l NAA, each combined with 2.3 mg/l BAP.

showing callus growth from single node cutting cultured on 5.0 mg/l,
Showing clearly the protuberance on the callus on 1.0 mg/l.

l. Showing the death stage of the two best maintained calluses obtained from leaf cultures on half-
ength modified MS medium.

) unviable callus on 1.0 mg/l NAA.
dead callus on 3.0 mg/l NAA.

The present work has shown that nodal explants responded well to treatment than the leaf cultures because callus growth was most prolific and without pronounced necrotic effects as observed with the callus derived from leaf explants on both half- and full-strength modified MS medium.

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