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STUDIES ON KOLA TISSUE CULTURE II: Effect of plant growth regulators on callus induction

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Abstract

Callus induction was studied on leaf and single node cutting explants obtained from *Cola nitida* (Vent.) Schott and Endlicher seedlings. Callus was induced successfully from cut surfaces (periphery) of young leaves *in vitro* on the medium supplemented with 0.5-5.0 mgl^{-1} naphtheneacetic acid (NAA), combined with 0.23 mgl^{-1} 6-benzyl-aminopurine (BAP) and on medium supplemented with 0.2-0.6 mgl^{-1} BAP, combined with 1.0 mgl^{-1} NAA. Successful callus induction was also obtained from the buds of single nodal explants cultured on MS medium supplemented with 0.1-1.0 mgl^{-1} NAA, combined with 2.3 mgl^{-1} BAP.

Introduction

The traditional method of kola propagation and germplasm conservation have always been through the raising of the seedlings from the nuts (seeds) and conservation in-situ and ex-situ of seeds and in orchards. Many problems have been implicated for low production level of kola. These include: the long juvenile period, low fruit/seed production, slow and uneven seed germination, sterile interspecific hybrids *C. nitida* and *C. acuminata,* and prevalent self- and cross-incompatibility of *C. nitida* trees (Jacob, 1971). Numerous studies have been conducted on the conventional methods for investigating problems of kola propagation. Several attempts have been made to induce uniform germination in *C. nitida* (Oladokun, 1985). Some studies on the sterile F_1 hybrids of kola have been reported (Jacob and Scott-Emuakpor, 1975; Morakinyo *et. al.;*1981). All vegetative methods of propagation [cutting, budding, marcotting (aerial layering) and grafting] have been practiced on kola (Egbe and Oladokun, 1987). However, the problem of low productivity still remains largely unsolved.

Adebona (1992) suggested the use of tissue culture studies as a means of overcoming some of these problems. The application of in vitro technologies has been employed for a large variety of trees, mostly temperate species (Kannan and Jasrai, 1996). Persley (1992) stressed the need for the applications of biotechniques to many tropical commodities with a view to resolving constraints to their productivity. However, successful application of tissue culture system depends soley on a judicious choice of variables including the explant type (Holme and Petersen, 1996); developmental stage and size (Eapen and George, 1990); growth medium, culture condition, growth regulator, among others (Brown, 1990). The optimization of such factors had led to successful callus formation and maintenance of callus cultures in many species (Xiao *et. al.*; 1997; Obembe *et. al.*, 1999). This paper presents the requirements for the survival and callus formation of kola tissues *in vitro.*

Materials and methods

Leaf callus induction

The basal medium used for these and subsequent experiments was modified Murashige-Skoog (MS) medium (Murashige and Skoog, 1962) (without Zn and Cu elements). Four different sets of auxin/cytokinin combinations were tested. The first two sets were supplemented with either of the

auxins -NAA or 2,4-dichlorophenoxyacetic acid (2, 4-D), at 0.5, 1.0, 3.0, and 5.0 mgl^{-1}, combined separately with 0.23 mgl^{-1} BAP. The range of NAA concentration tested for callus induction was adopted according to Lydiane (1983). The last two sets were supplemented with either of the cytokinins - BAP or 6-furfurylaminopurine (kinetin), at 0.2, 0.6, 1.0, and 1.5 mgl^{-1}, in combination with 1.0 mgl^{-1} NAA.

Nodal explant callus induction

The following combinations of auxin and cytokinin were tested: - NAA (0.1, 0.5, 1.0 and 1.5 mgl^{-1}), keeping BAP constant at 2.3 mgl^{-1} and BAP (0.6, 1.2, 1.8 and 2.4 mgl^{-1}), keeping NAA constant at 1.0 mgl^{-1}. Cultures of both explants were kept under observation for 4 weeks for callus induction.

Results and Discussion

Leaf callus induction

Callus induction was successful on NAA (0.0-5.0 mgl^{-1}) with 0.23 mgl^{-1} BAP (Table I). The callus obtained with a combination of NAA and BAP was creamy white in appearance (Fig. 1). There was no specific trend in the effect of the different NAA concentrations tested, except for the fact that callus induction was first observed with the medium supplemented with 1.0 mgl^{-1} NAA and 0.23 mgl^{-1} BAP. Callus induction on leaf explants of *Artemisia absinthium* was best with medium supplemented with 0.5 mgl^{-1} BAP and 2.3 mgl^{-1} NAA (Nin *et al.*, 1996). The callus obtained in 2,4-D and BAP was only observed after the 8th week as whitish sparsely distributed particles on the intact surfaces of the leaf explants. Sondahl and Sharp (1978) reported callus induction from *Coffea arabica* leaves using 2,4-D (1.1 mgl^{-1}) and kinetin (4 mgl^{-1}). Successful callus induction on the embryos of a tree species, *Tulipa gesneriana,* on medium supplemented with 2.2 mgl^{-1} 2,4-D alone has also been reported (Famelaer *et. al.* 1996*)*. Though, the same concentration range was employed for 2,4-D and NAA treatments in this work, satisfactory callus induction could only be achieved with NAA.

Table I: Summary of the separate effects of synthetic auxins (2,4-D and NAA) on callus induction from leaf explants.

Medium	Concentration (mgl^{-1})	2,4-D	NAA
Full modified MS medium	0.0	p g	** g 2
	0.5	* y 1	** g 4
	1.0	* y 2	** g 1
	3.0	* y 3	** g 2
	5.0	* y 4	** g 5

Key to observations
* = callus formed on the leaf surface
y = yellowish green coloration of the explant
g = explant still green
p = potentially good explant
** = induction of potentially viable callus
1-5 = order of occurrence of callus induction

Fig. 1: Leaf-derived callus

Combined effect of BAP and NAA, kinetin and NAA on callus induction on leaf explants are presented in Table II. Callus induction was successful on medium supplemented with 0.1, 0.2 and 0.5mgl^{-1} BAP. With kinetin however, no callus induction was observed, although the leaf cultures remained fresh after the 7th week. The retention of the green colour of the explant on kinetin could be due to cytokinin-delayed senescent effects (Salisbury and Ross, 1991).

Table II: Effect of cytokinins (kinetin and BAP) on callus induction from leaf explants

Medium	Concentration (mgl^{-1})	BAP	Kinetin
	0.0	* 1 g	p g
Full modified	0.2	* 2 g	p g
MS medium	0.6	* 3 g	p g
	1.0	* p g	p g
	1.5	* p g	p g

Key to observations
* = callus induction
p = potentially good explant
1-3 = order of occurrence of callus induction
g = explant still green

Induction of callus on nodal explants

Successful callus induction was achieved on explants implanted on 0.1-1.0 mgl^{-1} NAA in combination with 2.3 mgl^{-1} BAP (Table III). However, no successful attempts of callus induction was achieved with various levels of BAP in combination with 1.0 mgl^{-1} NAA (Table III). Single node explants have been found suitable for micropropagation of tree species (Kannan and Jasrai, 1996). Cardoso and Oliveira (1996) reported that single node explant callus can be induced in the presence of only one auxin, 2,4-D or NAA.

This work has been able to provide basic information on the requisites for callus induction on kola tissues. Further studies on callus growth on both explants continue.

Table III: Summary of the effect of plant growth regulators (NAA and BAP) on the induction of callus on single node cuttings.

Medium	Concentration (mgl^{-1}) NAA	NAA	BAP	Concentration (mgl^{-1}) BAP
	0.0	b	p	0.0
Full modified	0.1	** 3	+	0.6
MS medium	0.5	** 1	p	1.2
	1.0	** 2	p	1.8
	1.5	b	o	2.4

Key to observation
** = induction of potentially viable callus
p = potentially good explant
b = explant turns brown and dies
+ = contamination
o = sterilization damage on the tissue
1-3 = order of occurrence of callus induction

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