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RESEARCH ARTICLE 48

Embryogenesis and Plantlet Regeneration *via* Immature Male FlowerCulture of Banana (*Musasp.*)cv. *Grand Nain*

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(**Received** March 12, 2013; **Accepted** April 28, 2013)

***Abstract*— Somatic embryogenesis of banana was obtained from indirect embryogenesis of immature male flowers explants when cultured on MS basal medium supplemented with (0.5, 1.0, 2.0 and 4.0 mg/l of 2,4-D(Dichlorophenoxy acetic acid) . The calluses formed showed embryogenic characteristics, contained clusters of small tightly packed cells with dense cytoplasm, relatively large nuclei and very dense nucleoli. After 4 months of cultures on MS basal medium, somatic embryos started to regenerate. Presence of BA(Benzyl adenine)on theregenerationmedia increased *in vitro* shoot multiplication. Maximum number of suckers was obtained at 5.0 mg/l BA. For rooting 5.0 mg/l of kinetin (Kinetin 6 furfurylamino purine) gave highest number of roots. No morphological abnormalities were observed either in green house or after transplanting in the field.**

***Index Terms*— Banana, cv*GrandNain*, immature male flower, Embryogenic callu**

I. INTRODUCTION

Banana is the fourth world's most important food crop after rice, wheat and maize. It is a staple food and an export commodity which contributes to the food security of millions of people in the developing world and when traded in local markets provide income and employments to rural population. The fruits eaten as desserert without cooking are called bananas whilst the more starchy types wich need cooking before they can be used as vegetables are called plantains [1]. In the Sudan, Banana is the poor man fruit due to its relatively low price. Also it has been potential as an export crop particularly to the Arab countries. *Dwarf Cavendiish* is most widely grown banana cultivar in the Sudan but it is not suitable for export because it is high susceptible to black sigatoka and nematodes .Efforts to improve banana production in Sudan were started in 1994 with the introduction of new germ plasma e.g. *Grand Nain* and William [2].Since virtually all of the edible clones are parthenocarpic and for all practical purpose seedless or seed-sterile multiplication must be vegetative. Materials which can be used for propagation include bottom buds, suckers and bits. Despite the availability of these various propagates there has often been shortage of of suckers, the common planting material, The transmission of harmful nematodes and black sigatoka disease on field grown suckers necessitates the production of clean planting material and impedes the international transfer of germ plasma [3].Consequently attention has been drawn to the possibility of using tissue culture techniques for propagation [4].

Plant cell and tissue culture techniques have helped in the rapid multiplication of banana varieties employing shoot tips or floral apices [5]. Immature male flowers have been used to initiate cultures of several banana cultivars Escalant *etal*.,[6]Somatic embryogenesis has been reported from leaf sheath, rhizome fragments from *in vitro* plants[7], thin sections from proliferating buds[8], immature zygotic embryos[9]and male flower bud cultures[10]. Somatic embryos were obtained from callus derived from leaf base cultured in liquid media [11].However, these embryos regenerated only roots. The conversion of the embryos into plantlets is frequently low thus limiting its association with genetic transformation techniques [12].

The embryogenic culture system described in this paper demonstrates the potentiality of the male flower bud explant for use in raising highly proliferative embryogenic culture for banana cultivar *Grand Nain* and to study the effect of BA and Kin on plantlets regenerated from embryogenic callus.

II. MATERIALS AND METHODS

The fresh male flower cones were collected from *Grand Nain* plants at the time of complete bunch emergence. Outer enveloping bracts were removed and surfaces were cleaned with absolute alcohol. Under laminar airflow cabinet, the remaining outer enveloping bracts were removed until the inner part (2-3 cm in length) containing male flower primordial (from 8 to 16.0 being the meristem) was isolated.

Individual male flower primordia were excised and cultured on Murashige and Skoog's [13] basal medium (MS) supplemented with 0.5, 1.0, 2.0 and 4.0 mg/l 2, 4-D for initiation of callus.
The cultures were kept in total darkness under high humidity conditions (>70% R.H.) at 27 ℃. The cut surface was in contact with the medium. Containers were sealed with household foil. The male flowers were left on the same culture medium during the entire embryogenesis induction phase. The cultures were observed monthly during the first 3 months and every 2 weeks thereafter the calli were formed . For cytological observation, the tissues was fixed in FAA (formalin glacial acetic acid: 70%ethanol 5:5:90 v/v/v) ,dehydrated through a tertiarybutyl alcohol series and embedded in paraffin. Section were cut on a notary microtome and stained with safranin [14]. Concentrations of 1.25, 2.5 and 5.0 mg/l of BA were tested for successful culture establishment of shoot tips which were derived from the plantlets regenerated from callus. Kinetin (kin) was used in different concentrations (1.25, 2.5 and 5.0 mg/l) which were added separately to MS basal medium in order to study the effect of kin on morphogenesis of plantlets regenerated from callus.

III. RESULTS AND DISCUSSION

The response of 2,4-D medium on immature male flower was callus development. The higher concentration of 2,4-D(2.0-4.0mg/l) resulted in a rather friable consistency of the callus where at the lower concentration (0.5-1.0mg/l) the callus was relatively compact .After few subculture the tissues on the higher concentration became dark brown and disintegrated. Those on the lower concentration remained rather unchanged indicating that, these might probably be more suitable for the maintenance of embryos.
Male flower buds cultured on MS medium supplemented with 0.5-1.0 mg/l) of 2, 4-D exhibited swelling of the floral primordia within the first 3-4 weeks. After 5-6 months of male flowers culture, whitish callus was developed. The callus comprised compact, nodular embryogenic regions and non-embryogenic, loose, cellular mass. Results of cytological examination confirmed the presence of embryoids in E-callus. Some of the cells of the E-callus had dense cytoplasm (plate 1). The embryogenic callus often showed somatic embryogenesis at the early stages of embryo development. The embryogenic tissues showed good proliferation during subcultures on the induction medium. Somatic embryos transferred to MS basal medium developed further into plantlets (Plates 2, 3 and4).

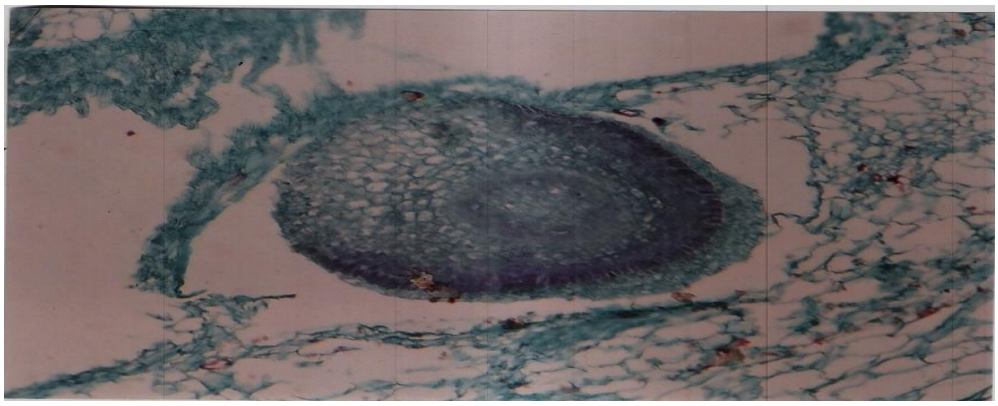

Plate1: Microtome section showing somatic embryo regenerated from male flower explants Culture donfull MS solid medium supplemented with 2, 4-D

Plate 2: male Flower explants cultured on induction media

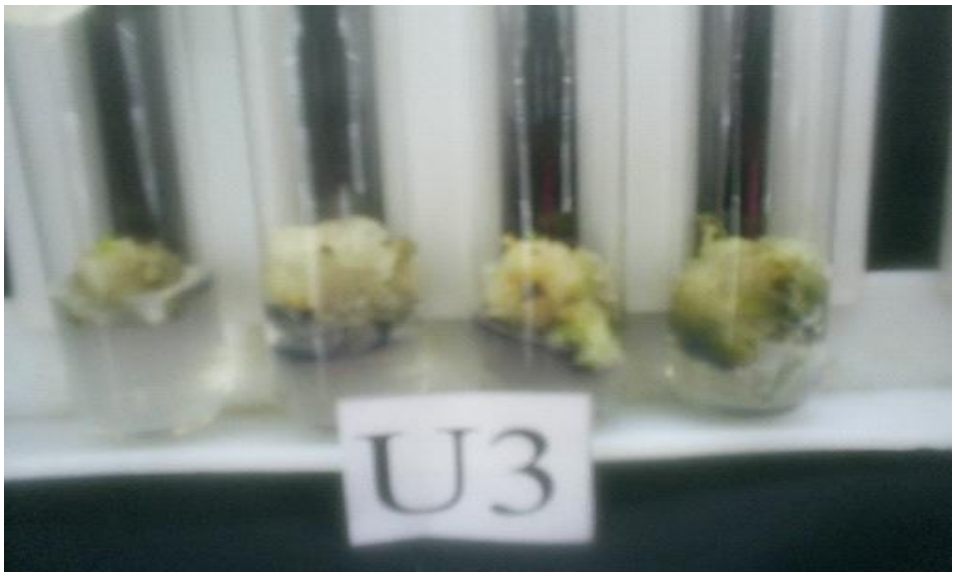

Plate 3: Initiation of callus on male flower explants

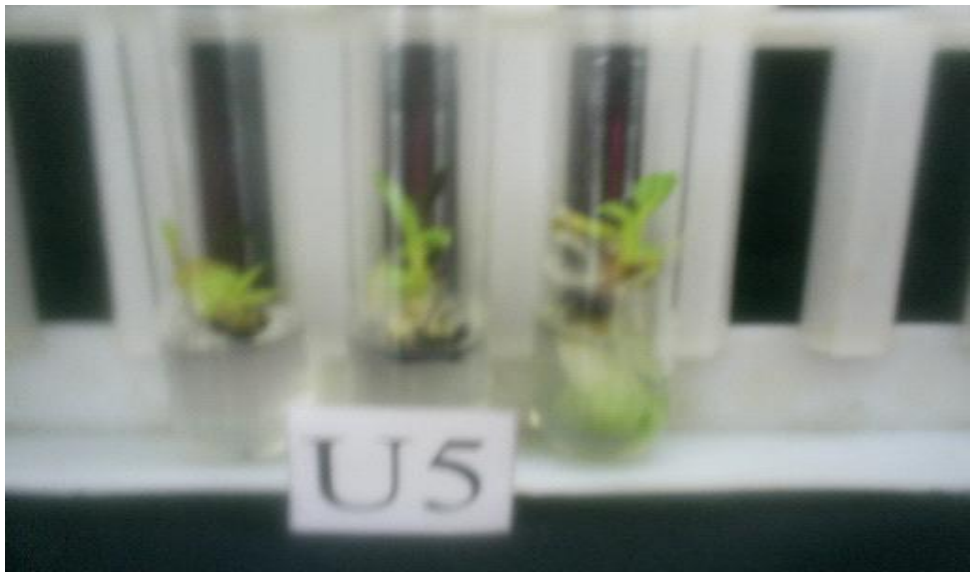

Plate 4: Regeneration of plantlets from somatic embryos

Presence of BA increased *in vitro* shoot multiplication of plantlets regenerated from embryogenic callus. The maximum number of suckers was obtained on 5.0 mg/l compared to 1.25 and 2.50 mg BA(**Table 1**). For rooting 5.0 mg/l of kin gave highest number of roots compared to 1.25 and 5.0 mg/l(**Table 2**). These plantlets were further established in the green house.

An effective system for somatic embryogenesis banana cv *GrandNain* has been developed based on young male flower. The reactivity of young tissues and particularly floral tissues is well known for numerous species [15].The possible use of low concentration of 2,4-D enhanced the formation of embryogenic callus. There were some observation such as that in the literature [16].The development of embryos into plant took place when these embryos were transferred from 2,4-D to MS basal media. Results such as that are showed [17],[18].Generally reduction of auxin concentration or it is complete omission leads to the germination of somatic embryos. The presence of BA was necessary for banana colonial propagation since cytokinins induce multiple shoot formation by supporting apical dominance of the main meristem[19].The highest number of leaves and suckers were achieved by using 5.0 mg/l of BA, Similar results were obtained by Cronauer and Krikorian[20] .Also this study indicated that the presence of Kin in MS basal media promoted rooting, This results consistent with literature [21] while Upadhyaya[22] found that adventitious root formation occurred only in the presence of NAA(Naphthaline acetic acid).

Table (1)
Effect of BA (mg/l) on growth parameters of banana plantlets regenerated from embryogenic callus of male flower explants on MS after 8 weeks of incubation.

BA (mg/L)	**Growth parameters**			
	No. of leaves	**No. of suckers**	**No. of roots**	**Plant height (cm)**
0.00	2.63^{c}	1.50^{b}	2.38^{a}	1.26^{ab}
1.25	3.38^{bc}	2.13^{b}	1.50^{b}	1.44^{a}
2.50	3.88^{ab}	2.50^{b}	1.13^{bc}	1.16^{b}
5.00	4.75^{a}	4.38^{a}	1.00^{c}	1.21^{b}

Means having different superscript letters in the same column differ significantly (P ≥0.05) using Duncan's multiple range tests

Table (2)
Effect of kin (mg/L) on growth parameters of banana plantlets regenerated from male flower explants cultured on MS after 8 weeks of incubation.

Kin (mg/l)	**Growth Parameters**			
	No. of leaves	**No. of suckers**	**No. of roots**	**Plant height (cm)**
0.00	8.75^{a}	1.25^{a}	1.50^{c}	4.25^{a}
1.25	6.13^{b}	1.13^{a}	7.25^{b}	3.43^{b}
2.50	5.25^{bc}	1.00^{a}	9.50^{ab}	3.29^{b}
5.00	4.75^{c}	1.00^{a}	12.25^{a}	4.55^{a}

Means having different superscript letters in the same column differ significantly (P ≥0.05) using Duncan's multiple range tests

VI. CONCLUSION

As somatic embryogenesis is well established method in case of banana, the large number of banana genotypees still need to be screened for exploring their embryogenic potential for mass production and genetic improvement. In conclusion, this research demonstrates that plant regeneration via callus culture of immature male flower of banana cv *GrandNain* corresponds to an organogenesis through the development of induced embryogenically determined cells. It seems that2,4-D plays an important role in the induction medium. The presence of auxins (BA and Kin) is necessary for plant maturation. Developing a protocols for in vitro plant regeneration may aid efforts in the selection of disease resistance clones and transgenic plants with desirable traits aimed at the development of banana in Sudan.

ACKNOWLEDGEMENTS

We thank Khartoum University for financial supports. We thank Date palm technology company. Shambat.Sudan, where the practical of this study was carried.

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