Pak. J. Biotechnol. Vol. 2 (1-2) 36-40 (2005) ISSN. 1812-1837

STUDY OF CALLUS INDUCTION IN BANANA (Musa sp)

Abdullah Khatri, Imtiaz Ahmed Khan, Muhammad Umar Dahot*,Ghulam Shah Nizamani, Muhammad Aquil Siddiqui, Saboohi Raza, and Mazhar H.Naqvi

Nuclear Institute of Agriculture (NIA), Tando Jam, *Institute of Biotechnology & Genetic Engineering, University of Sindh, Jamshoro, Sindh, Pakistan E-mail: niatjam@hyd.paknet.com.pk

ABSTRACT:

Proembryo calli were produced from basal sheath and rhizome tissue excised from multiplying shoot clusters in cvs. SH 3362 and GN 60A (AAA). SH medium with 30 µM/l 3,6 dichloro-2 methoxybenzoic acid and 5µM/l thidiazurone showed best results. Browning of explant was reduced by addition of cystein and methioninne. Light and dark treatments were given to reduce the browning in the explant

INTRODUCTION:

Banana and plantains (Musa ssp) are among the world's most important crops, and is major food staple in many parts of the tropics and subtropics. The clones of commerce are triploid and all practical purposes seed sterile. Therefore, propagation is necessarily by vegetative means. Efforts to improve banana using conventional breeding techniques produced very limited results (Rowe and Richardson, 1975; Rowe, 1984). Breeding of new banana varieties by sexual crossing is extremely difficult because only a few diploid clones produce viable pollen to use in crosses with female fertile triploids for the production of a new tetraploid seed (Shephered, 1987; Rowe and Richardson, 1975).

Consequently, attention has been drawn to the possibility of using aseptic culture techniques to induce variation and to recover novel plants (Krikorian and Scott, 1990). Somatic embryogenesis is the process by which haploid or diploid somatic cells develop into differentiated plants through characteristic embryological stages without fusion of gametes. Currently, somatic embryo genesis is best known as a pathway for induced regeneration from in vitro tissue culture occurring indirectly from callus or directly from cells of an organized structure. Mohan Ram and Steward (1964) initiated callus proliferation and slow growing cell suspension from immature fruit tissue, however these cultures proved to be non-morphogenic. The formation of spherical callus masses in Musa tissue culture resembling somatic embryos were observed by Srinivasan et. al., (1982); Cronauer and Krikorian (1983); Cronauer and Krikorian (1986); Jarret et. al., (1985) and Novak et. al., (1993). While somatic embryos and subsequently recovery of plantlets was observed in zygotic embryos of M.Ornata (Cronauer and Krikorian, 1988).

In this communication, we have also describe the production of callus and somatic embryos from in vitro cultured shoots of two banana cultivars having genome AA and AAA.

MATERIAL AND METHODS:

Multiplying shoot culture of Musa cultivars SH3362 (AA) and GN 60A (AAA) was established according to procedure described earlier (Khatri et al. 1997). Basal part of rhizome tissue and leaf sheaths were excised from multiplying cultures of both the clones for initiation of callus induction and somatic embryos and placed on macro and micro elements of Schenk and Hildebrandt (SH) medium (1972). The medium was supplemented with staba vitamins, inositol 100 mg/l, cystein HCl 40 mg/l, sucrose 4% and growth regulators. The media was adjusted to pH 5.8 and solidified with 0.2% gelrite. Growth substances Dicamba (3,6 dichloro-2 methoxybenzoic acid) and Thidiazurone at concentration of 30 μM/l and 5μM/l were used respectively. Besides, effect of light and darkness on browning and callus induction was also noted.

RESULTS AND DISCUSSION:

It was observed that rhizome tissue taken from the central cylinder of corms proliferated proembryo-genic callus on SH media containing staba vitamins and dicamba at 30μ M/l concentrations within 3-4 weeks of explantation. By comparing 4 different media it was observed that in D2B cys and D2B met corm and leaf sheaths produced white, friable and compact callus than DB cys and D2B cys met in both the clones (Fig 1 and 2).

Excised banana tissue also manifested rapid and very severe discoloration, eventually producing an intensely black medium as well as tissue and callus. Combination of 30 μM/l dicamba and 5 μM/l thidiazurone improved callus formation and proved worth to overcome the problem of browning of tissue and medium (Novak et al., 1983). The use of cystein HCl (Khatri et al.1997) and methionine at 40 mg/l concentration either in combination or alone avoided browning but combination showed better performance in improving callus formation. Problem of blackening of the medium was resolved by replacing agar with gerlite as a gelling agent. Light showed positive correlation with browning, explants when incubated in the dark showed less browning as compared to the explants incubated in light. This might be due to higher physiological activity, which is more under light conditions as compared to dark (Table 1).

Both the genotypes showed different proliferation patterns from rhizome explants. It was observed that ploidy have some impact on the callus formation in banana because diploid clone SH3362 (AA) yielded both callus and cylindrical proembryonal structures whereas, triploid clone GN60A (AAA) formed semi-compact callus with rare proembryonal structure. This also confirms that callus induction is genotype dependent phenomenon in banana. It was observed that diploid clone SH3362 yielded more callus when grow on media containing dicamba as compared to triploid GN60A. No shoot formation was achieved when pro-embryogenic and somatic embryos transferred to regeneration medium.

In conclusion, formation of somatic embryos using immature leaf segments as described in the present study, could be useful in creating new

genetic diversity or it may allow improving the existing cultivar by altering one or few traits keeping the whole genome intact. Further, such a system should allow transformation methods like *Agrobacterium* or particle bombardment to be more successful by giving rise to more transformed plants in a short time.

Table 1: Effect of dark/light on callus/embryogenic calli formation and browning in two *Musa* clones SH 3362 (AA) and GN60A (AAA)

Media	No.of Explants	Callus %	Embryoid formation	Browning
a) Effect of dark on SH 3362 (AA)				
DB Cyc	42	52	+++	++
D2B Cyc	65	63	+	+
D2B Met	60	60	+	+
D2B Cys Met	69	68	++	+
b) Effect of dark on GN60A(AAA)				
DB Cyc	36	30	++	+++
D2B Cyc	78	17	+	++
D2B Met	28	28	+	+
D2B Cys Met	44	36	+	+
c) Effect of light on SH 3362 (AA)				
DB Cyc	46	89	+++	+++
D2B Cyc	37	89	+	++
D2B Met	37	86	+	+
D2B Cys Met	35	88	++	+
d) Effect of light on GN60A(AAA)				
DB Cyc	27	37	++	+++
D2B Cyc	34	21	+	++
D2B Met	26	38	+	++
D2B Cys Met	24	42	+	++

DB Cys = SH + Dicamba + Cystein HCl , D2B Cys = SH + Dicamba + Cystein HCl + Thidiazurone, D2B Met = SH + Dicamba + Methionine + Thidiazurone, D2B Cys Met = SH + Dicamba + Cystein HCl + Thidiazurone + Methionine

Embryoid: + = Fair to poor, ++ = Good, +++ = Excellent

Browing: + Few, ++ = Moderate, +++ = Substantial

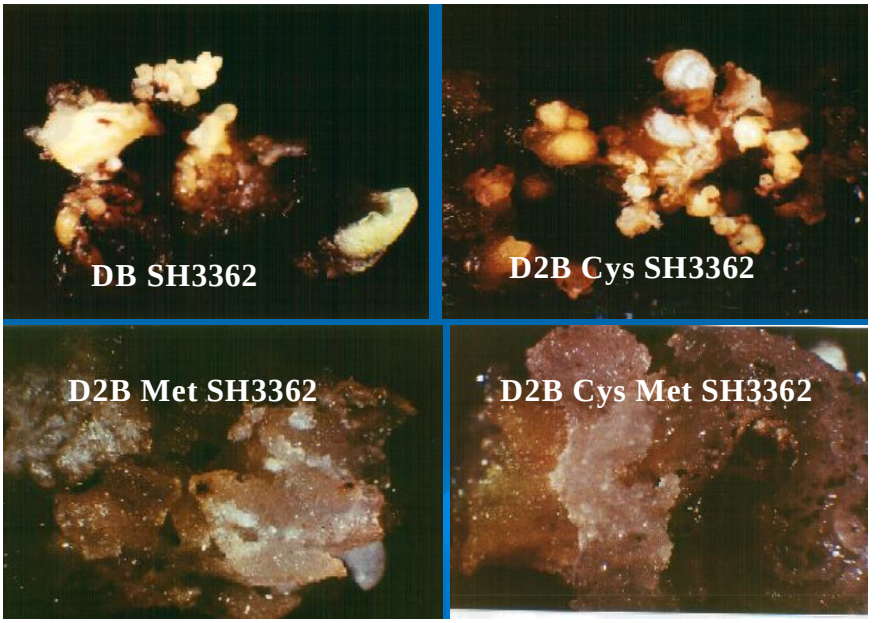

Fig.1. Callus and embryoid formation in SH3362

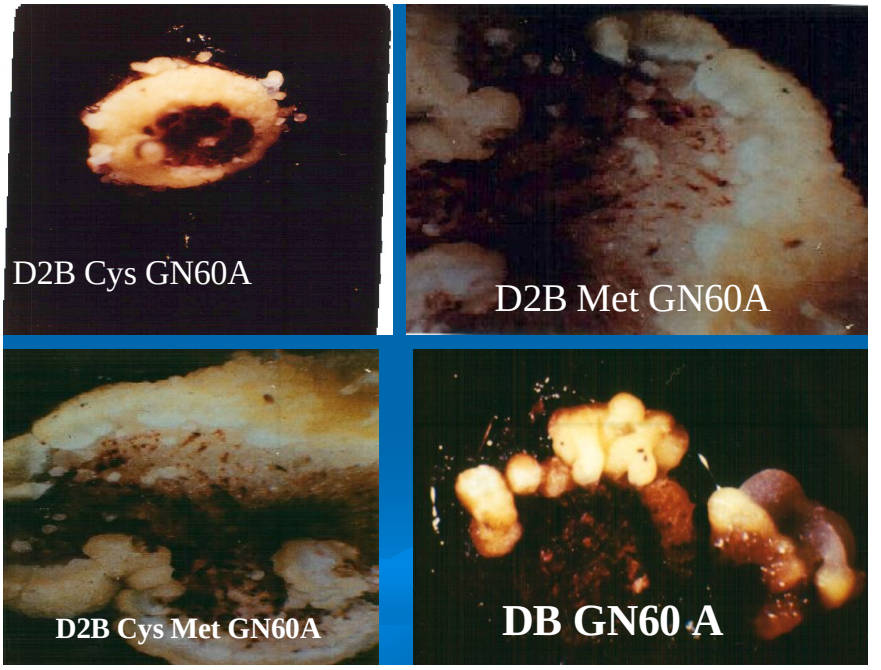

Fig. 2. Callus and embryoid formation in GN60A

REFERENCES:

Cronauer, S.S. and A.D. Krikorian, Somatic embryo from cultured tissue of triploid plantains (Musa ABB). Pl. Cell Rep. **2**: 289-291 (1983)

Cronauer, S.S. and A.D. Krikorian, Regeneration in bananas and plantains In: Vasil IK (ed) Cell Culture and Somatic Cell Genetics of Plants, Academic Press, Orlando. 3:179-186. (1986)

Cronauer, S.S. and A.D. Krikorian, Plant regeneration via somatic embryogenesis in seeded diploid Musa ornate Roxb. Pl. Cell Rep. **7**:23-25 (1988)

Jarret, R.L., J.B. Fisher and R.E. Litz, Organ formation in *Musa* tissue culture. J. Plant Physiol. **121**: 123-130 (1985)

Khatri, A., I.A. Khan, S.H. Siddiqui, A. Ahmad and K.A. Siddiqui, In vitro culture of indigenous and exotic banana clones for maximising multiplication. Pak. J. Bot. **29**(1): 143-150. (1997)

Krikoren, A.D. and M.E.Scott, Musa callus and cell culture: Strategies, achievements and directions. In *In-vitro* mutation breeding of banana and plantains I. IAEA, Tec. Doc. Vienna, Pp 7-23. (1990)

Mohan Ram, H.Y. and F.C. Steward, The induction of growth in explanted tissue of the banana fruit. Canad. J.Bot. **42**:1559-1579 (1964)

Novak, F.J., H. Brunner, R. Afzal, R.K. Morpurgo, M. Upadhyay, M. Van Duren, Sacchi, J. Sitti, A. Hawa, Khatri, G. Kahl, D. Kaemmer, J. Ramser, and K. Weising, Improvement of Musa through biotechnology and mutation breeding. In. Proc. Biotechnology for banana and Plantain Improvement. San Jose, Costa Rica, Inibap. pp.143-158. (1993)

Rowe, P., Breeding banana and plantains. Plant Breeding Reviews **2**:135-155 (1984)

Rowe, P. and D.L. Richardson, Breeding banana for diseases resistance, fruit quality and yield Bulleitin No. 2. Topic. Agric. Res. Ser. Hondurs Pp.1-44. (1975)

Schenk, R.U. and A.C. Hildebrandt, Medium and techniques for induction and growth for monoc-otyledonous and dicotyledonous plant cell cultures. Canad. J. Bot. **30**: 199-204 (1972)

Shephered, K. Banana breeding: Past and present. Acta Horti. **196**: 37-43 (1987)

Srinivasan R.N.K, E.K. Chacko, R. Dore Swamy ad E. Narayana Swamy. Induction of growth in explanted inflorescence axis of banana. Current Sci. **51**: 666-667 (1982)