Pak.J.Bot., 29(1): 143-150, 1997.

IN VITRO CULTURE OF INDIGENOUS AND EXOTIC BANANA CLONES FOR MAXIMISING MULTIPLICATION

ABDULLAH KHATRI, IMTIAZ AHMED KHAN, SHAMIM H. SIDDIQUI, MAQBOOL AHMAD AND K.A. SIDDIQUI

Atomic Energy Agricultural Research Centre, Tando Jam, Sindh, Pakistan.

Abstract

Meristematic tip with two pairs of leaf primordia from 5 clones of dessert banana (*Musa* spp.) viz., GN60A, SH3362 William, Highgate and Basrai were evaluated for *in-vitro* propagation. Rapid shoot proliferation cultures were established in MS medium with 20μM BAP. Clone GN60A produced highest tillers followed by clone SH3362, with lowest number of tillers produced by Clone Basrai. Shoots were easily rooted on MS medium with 4% sucrose. Oxidation of phenolic compounds was controlled by addition of 40 mg/l cystein HCl

Introduction

The propagation of banana is practiced by vegetative means because it is one of the most conspicuously sterile crops of the world (Rowe, 1984). Propagation of banana by conventional methods has been described by many workers (Ascenco, 1967; Hamilton, 1965; Barker, 1959; Simmonds, 1966), however, these techniques are laborious and time consuming for the production of homogeneous plants. Moreover the rate of propagation of *Musa* plants by these methods is rather low since only 4-5 plants per year from single sucker can be obtained whereas 1000 plants are required for one acre plantation. The low multiplication ratio of banana has promoted intensive research for rapid propagation methods and attention has been drawn to the possibility of using aseptic culture techniques which facilitates quick multiplication in higher quantity.

In recent years rapid spread of banana bunchy top disease (BBTD) in the province of Sindh has adversely affected the banana cultivation where the farmers have reduced banana cultivations by upto 60% and production decreased by upto 90% (Khushk *et al*; 1993). BBTD incidence in some area was up to 100%. The banana cultivation has therefore been stopped in many areas and the land has been devoted to other crops (Jones, 1992). Thus availability of disease free plants has become a major problem for banana cultivation in Sindh. For the renaissance of this crop, rapid multiplication of disease free plants as well as better agronomic practices are needed. Therefore new strategies including implementation of biotechnology may be a suitable approach to achieve this goal. Advances so far in *in vitro* culture methodologies i.e., micropropagation and their applications have made significant contribution (Gupta, 1986; Dore Swamy & Shijram, 1989; Cronauer & Krikorian, 1984; Novak *et al.*, 1989, 1990, 1993; Jarret *et al.*, 1985; Siddiqui *et al.*, 1991; Khatri *et al.*, 1996; Drew *et al.*, 1992; Gardner, 1993) to resolve these challenging problems, hence

Fig 1. Banana explant.

micropropagation of banana shoot tip has lead to rapid multiplication and virus elimination (Drew *et al.*, 1992).

The aim of the present study was to apply and simplify the existing *in vitro* culture methods to banana cultivars to produce *in vitro* plants under modest local technical conditions and to obtain plants free from pathogens.

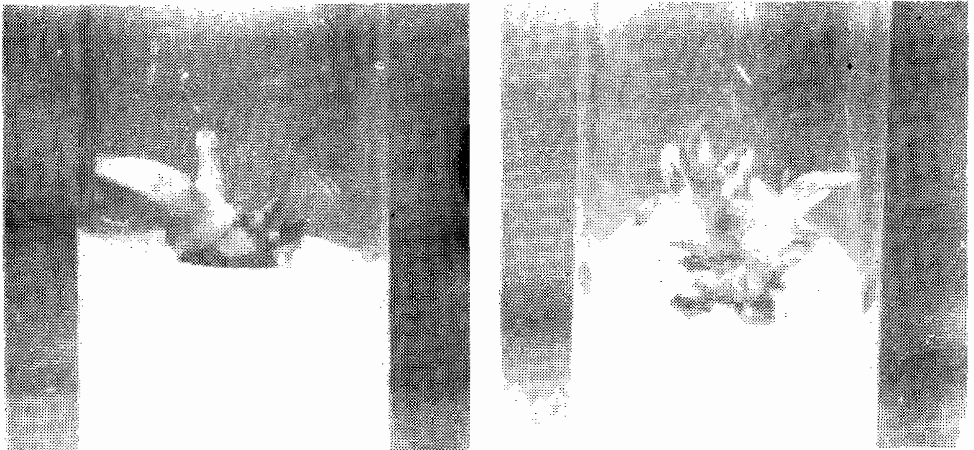

Fig 2. Shoot multiplication in banana.

Materials and Methods

The work has been developed with cultivars Dwarf Cavendish Basrai (commercial clone of Sindh), William hybrid, GN 60A (mutant of Grand Nain), SH 3362 and Highgate, a spontaneous mutant. The *in vitro* material was provided by Maroochy Horticultural Research Institute, Australia and IAEA Seibersdorf Lab. Vienna, Austria. The explants obtained from young developing suckers or corms consisting of shoot tips and rhizomatous base were thoroughly washed in tap water and surface sterilized with 70% ethanol for 60 seconds followed by immersion in 5% sodium hypochlorite for 20 minutes and rinsed thoroughly in sterilized distilled water. More leaves were then peeled off until the remaining shoot tips left were approximately 1cm at the base. These were cut into four equal pieces and each piece explanted in culture test tube containing Murashige & Skoog (1962) modified medium and incubated at 25 $\pm$ 2°C under 16 h photoperiod (Fig.1).

The culture medium used were:

a) Initial medium MS + 40 mg/l Cystein HCl + 5 uM/l IAA + 10 uM/l BAP + 40 gm/l Sucrose
b) Multiplication medium MS + 40 mg/l Cystein HCl + 20 uM/l BAP + 40 gm/l Sucrose
c) Rooting medium 1/2 MS + 1 uM/l IBA + 40 gm/l Sucrose

The plants after root development were acclimatized and transplanted into soil.

Results and Discussion

a) Establishment phase: The colour of survived explants changed from creamy white to green within 7-10 days. Shoot tips initially cultured on solid medium showed tendency to produce phenolic compounds in all clones tested which oxidized rapidly and resulted in the death of the explants. The use of cystein HCl 40 mg/l in the medium proved most effective to prevent excessive production of polyphenolic compounds and increased the survival rate of explant. The degree of blackening of the culture medium was considerably reduced after a few subcultures. Shoot tips were established on solid MS medium with 10μM/l BAP and 5μM/l IAA. One to two shoots were produced after 14-21 days of initial culture (Fig.2). Dissection of these shoot tips in subsequent culture stimulated shoot multiplication upto 7-10 shootlets in MS medium with BAP 20 μM/l (Fig.3).

b) **Multiplication** phase: At low concentration of BAP (10 μM/l) only one or two shoots were regenerated in most of the cultivars while at higher concentration (20 μM/l), multiplication of shoot bud was noticed with concomitant suppression of the shoot elongation (Fig 4). Addition of BAP at 20 μM/l concentration was most effective for the production of shoots. Splitting of shoot tips strongly induced multiple shoot formation (Siddiqui *et al*, 1991; 1993). Previous reports on the importance of apical dominance in shoot production from explant of banana are conflicting. Ma & Shii (1972) reported that the destruction of apical dominance by removing the domes was essential for the production of multiple shoot-initials in cv Cavendish. Swamy *et al*.,

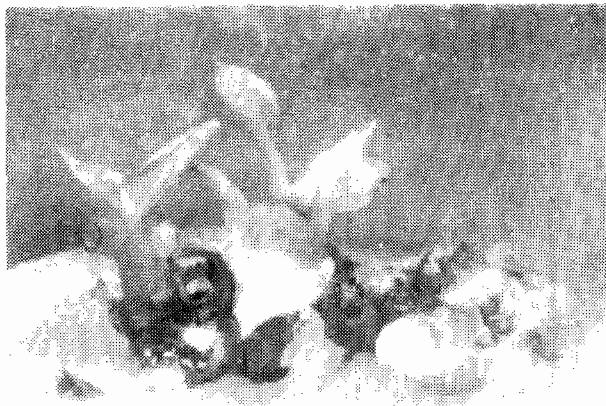

Fig.3. Shoot elongation in banana.

(1983) reported that multiple initials were produced in the presence of apical domes in cv. Robusta. The findings reported here for a range of cultivars support those of Swamy *et al.*, (1983) in that the removal of apical dome is not essential for multiple shoot formation.

Reculture of split shoot tip in liquid medium strongly increased the growth rate and shoot proliferation as compared with solid medium of the same composition (Fig.5 a,b). Alvard *et al* (1993) observed that temporary immersion in the liquid medium for 20 minutes gave the highest multiplication rate after 24 hours. In the present study, shoots of GN 60A when placed in liquid medium produced an average of 9 shoots per cluster with 4 shoots in solid medium. Similarly cv SH 3362 produced 8 shoots per cluster in liquid medium and 4 shoots in solid medium per cluster in 2nd sub- culture.

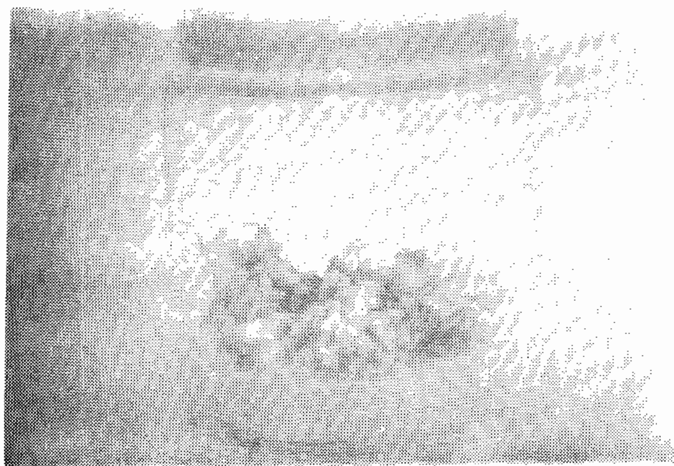

Fig.4. Suppression of the shoot elongation in banana.

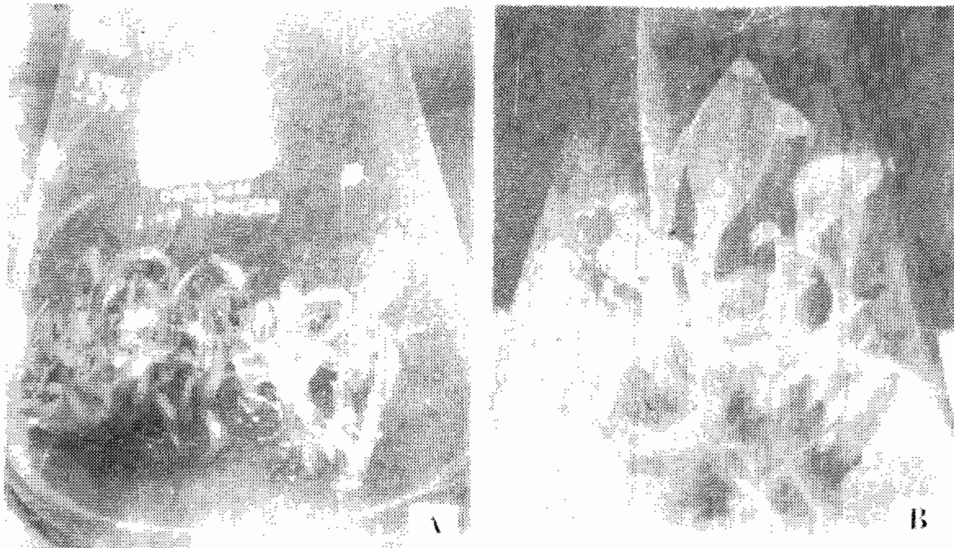

Fig.5 Shoot multiplication of banana in A = Liquid medium B = Solid medium

The morphogenetic responses of the shoot meristem tip as influenced by cultivars and number of subcultures are presented in Table 1. Different cultivars showed variation in the degree of shoot bud proliferation . The type of multiple budding appeared to be linked to genomic configuration. In general, two types of multiple budding could be distinguished: a) proliferation of "bulbils" and b) proliferation of "plantules". Differences in the proliferation rate was observed in all cultivars tested.

Highest multiplication was observed in GN60A followed by SH3362 with lowest multiplication found in Basrai indicating that multiplication is a genotype dependent phenomenon. Ten explants of GN60A produced 3600 plantlets in 3 months with the

Table 1. Multiplication rate of *Musa* cultivars studied *in vitro* in different cycles of cultures.

Clones	No. of explant	Multiplication rate: Original culture			1st sub-culture			2nd sub-culture		
		Ave.	Range	Total	Ave.	Range	Total	Ave.	Range	Total
GN 6OA	10	05	3-8	50	08	6-10	400	09	8-12	3600
SH3362	10	04	3-6	40	06	3-07	240	08	6-10	1920
William	10	04	3-5	40	04	3-07	160	05	3-07	750
Highgate	10	03	2-5	30	05	3-07	150	05	4-07	750
Basrai	10	03	2-4	30	03	2-04	90	03	1-04	180

result that in one year 14400 plantlets can be produced to cover 14 acres of land which otherwise is impossible through conventional practices. Interestingly, micropropagated plantlets are disease free and they did not encounter any biotic or abiotic stresses during their growth period whereas plants obtained through conventional methods may face such problems during their growth period.

c) **Rooting phase:** Root formation could be induced in a number of ways in about 4 days. However, root formation was also observed in multiplication medium but strong rooting system was accomplished only in rooting medium containing 1/2 strength MS medium with 1μM/l IBA and 40 gm/l sucrose within 7-10 days (Fig.6) as also reported by Siddiqui *et al.*, 1993). Different rooting behaviour was observed in liquid and solid medium. Shootlets placed in liquid medium developed profuse rooting with primary, secondary and tertiary rooting system. In solid medium only primary root was observed due to which plantlets obtained through liquid medium get established easily in the field as compared to the plantlets obtained from solid medium.

Transplantation to field: The plants with well developed root system were transferred to jiffy pots containing perlite and then to earthen pots (Fig.7). They were irrigated with distilled water daily and provided with liquid MS medium twice a week. After 1-2 weeks of incubation they were exposed to natural conditions for 8-12 weeks in the pot culture house. After acclimatization the saplings were transplanted in the field where the plantlets were found to grow vigrously in the field and did not show any symptoms of BBTD.

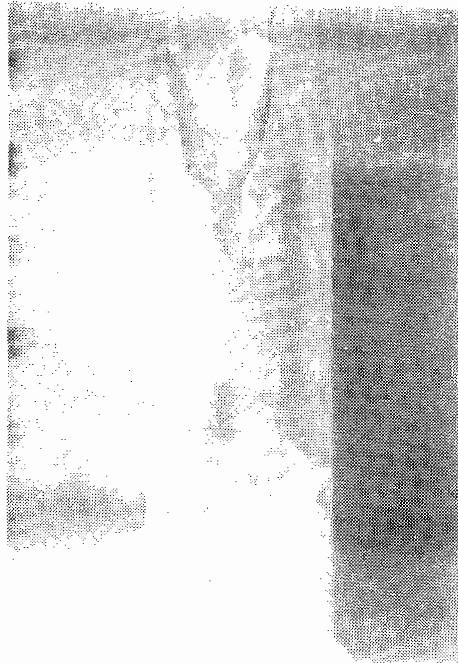

Fig. 6: Rooting in banana

Fig.7 Banana plantlets in earthen pots ready for transfer to field.

References

Ascenco J.C. 1967 A simple technique for the multiplication of banana planting material *Trop. Agric. (Trinidad)*, 44 243-244

Alvard D F Cote and C Teisson 1993 Comparison of methods of liquid medium culture for banana micropropagation Effect of temporary immersion of explant. *Plant Cell,Tissue and Organ culture*, 32: 55 60

Barker, W G 1959 A system of maximum multiplication of banana plant. *Trop. Agric.*, 36: 275-284.

Cronauer, S.S. and A.D Krikorian 1984. Multiplication of *Musa* from excised stem tips. *Annal. Bot.*, 53.321 328

Dore Swamy, R and L. Sinjram 1989. Micropropagation of banana from male floral apices cultured *in vitro* *Scientia Hortic.* 40: 181-188

Drew, R A , M K Smith and D.W. Anderson 1992. Field evaluation of micropropagated bananas derived from plants containing banana bunchy top virus. *Plant Cell Tissue and Organ Culture*, 28:203-205.

Gardner, R.C 1993 Gene transfer into tropical and sub-tropical crops. *Scientia Hortic.*, 55: 65-82.

Gupta, P. 1986. Eradication of mosaic disease and rapid clonal multiplication of banana and plantains through meristem tip culture. *Plant Cell Tissue and Organ Culture*, 6: 33-39.

Hamilton K S 1965. Reproduction of banana from adventitious buds. *Trop. Agric. (Trinidad)*, 42:69-73.

Jarret, R.L. W. Rodrignze and R Fernandez. 1985. Evaluation, tissue culture propagation and dissemination of Saba and Pelipita plantains in Costa Rica *Scientia Hortic.*, 25.137 147.

Jones, D. 1992. *Crop protection Annual Report 1992*, INIBAP, pp. 29-32.

Khatri, A., S.H. Siddiqui, I.A. Khan, K.A. Siddiqui, G.S. Nizamani, M.H. Khanzada and N.A. Dahar. 1996. A tissue culture technique for banana improvement. *In: New Vistas in Plant Genetics* (In Press).

Khushk, A.M., M.Y. Memon, R.A. Memon, M.I. Lashari and A.M. Khaskheli. 1993. Banana pests and diseases in Sindh *Modern Agriculture*. August pp 17-21.

Ma, S.S. and C.T. Shii. 1972. *In vitro* formation of adventitious buds in banana shoot apex following decapitation. *J. Hort Soc. China*, 18: 135-142.

Murashige, T. and F. Skoog. 1962 A revised medium for rapid growth and bioassays with Tobacco tissue culture. *Physiol. Plant* 15: 473-497

Novak, F.J., R. Afza, M. Van Duren, M. Perea-Dallos, B.V. Conger and X. Tang. 1989. Somatic embryogenesis and plant regeneration in suspension culture of desert (AA and AAA) and cocking (ABB) banana (*Musa* spp.) *Biotechnology*, 7: 154-159.

Novak, F.J., R. Afza, M. Van Duren, and M.S. Omar. 1990. Mutation induction by gamma irradiation of *in vitro* cultured shoot tips of banana and plantain (*Musa* ssp.) *Trop. Agric.* (Trinidad) 67 (2) 28

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

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

Siddiqui, S.H., A. Khatri, I.A. Khan, G.S. Nizamani and R. Khan. 1993. Improvement of banana (*Musa* spp.) through *in vitro* culture technique and induced mutations. In: *Proc. IAEA co-ordinated research programme of banana and plantains, IAEA, Vienna, Austria*.

Siddiqui, S.H., A. Khatri, I.A. Khan and M.H. Khanzada. 1991. Improvement of banana (*Musa* spp.) through *in vitro* culture techniques and induced mutations. In. Proc. 2nd. IAEA co-ordination meeting, San Jose, Costa Rica.

Simmonds, N.W. 1966. *Bananas*, 2nd ed. London, UK Longman pp. 512.

Swamy, R.D., N.K.S. Rao and E.K. Chacko. 1983. Tissue culture propagation of banana. *Sci. Hort.*, 18: 247-252.

(Received for publication 14 July, 1996)