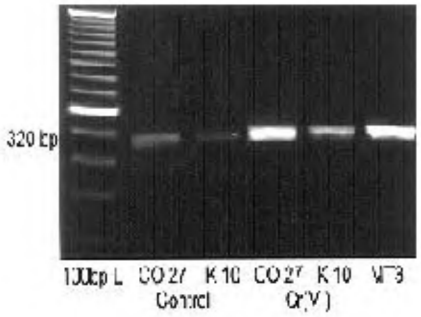

Figure 2. RT–PCR products for *MT3* gene in root tissue of sorghum cultivars K 10 and CO 27. Untreated control are treated with 100 µm Cr(VI) for 5 days in nutrient medium.

designed for *A. thaliana* MT3-like protein on the basis of the cDNA sequence of the gene obtained. The cDNA sequence of the gene was uploaded to the server (http://www.bibiserv.tekhfak.unibielefeld.de) of Gene Fisher on-line primer construction site (Query ID_1041256020_12071) with the following primer design specifics: Maximum primer size: 15 to 18 bp; primer GC content: 65 to 45; melting temperature: 42 to 55°C and PCR distance: 300 to 500.

The first of the top three primers returned was taken up for construction. Forward primer: GTCAAGCAACTGCGGAA, Reverse primer: GAAGGGAAAGAAGGGTCA and Product length: 319 bp. The products were analysed by electrophoresis in agarose gel by choosing the gel matrix for 320 size of the amplification product. RT–PCR products (Figure 2) were seen in all the samples, which suggests that RNA expression of this gene is present in sorghum. Similar results have been shown by Butler and Roesijadi[5], wherein the authors have reported that transcripts of two *MT3* genes accounted for an additional 1.25% of the mRNAs in rice. Comparison of band intensities of all the samples with that of *MT3* gene showed that there could be a varying degree of transcript abundance in the samples. From the results obtained it was seen that sorghum cultivar CO 27 treated with Cr(VI) had high-intensity band matching that of the gene of interest. This suggests that there could be higher transcription rates of the MT under Cr stress, particularly in the tolerant variety. It is possible that reactive oxygen species and H_2O_2 produced under Cr stress acted as a signal to induce MT mRNA transcription. Phytochelatin functions in the regulation of essential metals and in the detoxification of most toxic metals. The distinct absence of phytochelatin has been reported in plants under Cr(VI) stress[7]. This suggests that there could be an enhanced role for MTs in plants under Cr stress. There is a possibility that MTs could confer tolerance to plants against metal stress by binding Cr ions and rendering them non-toxic. A clear role for MTs is yet to be established, although they certainly are thought to play a role in metal metabolism. MTs may function as antioxidants and a role in plasma membrane repair is another possibility. Although MTs are expressed ubiquitously and conserved in plants, determining their function remains a future challenge.

1. Liu, J. Y., Lu, T. and Zhao, N. M., *Acta Bot. Sin.*, 2000, **42**, 649–652.
2. Palmiter, R. D., *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 8428–8430.
3. Murphy, A. and Taiz, L., *Plant Physiol.*, 1995, **109**, 1–10.
4. Kaplana, L. A. E., Kathleen, V. C., Virgina, I. and Joseph, F. C., *Mar. Environ. Res.*, 2000, **39**, 137–141.
5. Butler, R. A. and Roesijadi, G. *Aquat. Toxicol.*, 2001, **54**, 59–67.
6. Matsumura, H., Nirasawa S. and Terauchi, R., *Plant J.*, 1999, **20**, 719–726.
7. Toppi, L. S. D., Fossati, F., Musetti, R., Mikerezi, I. and Favali, M. A., *J. Plant Nutr.*, 2002, **25**, 701–717.

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Establishment of cell-suspension cultures in banana cv. Grand Naine and evaluation of its sensitivity to gamma-irradiation

Members of the family Musaceae (bananas and plantains) have a major contribution towards the world's total food production[1] and are an important staple food for millions of people inhabiting the humid and sub-humid tropics[2]. These are amongst the world's leading fruit crops, with an annual global production of about 88 million metric tonnes from an area of approximately 10 million ha. More than 100 countries throughout the tropical and sub-tropical regions cultivate bananas[3] and India is the world's largest banana producer[4].

Musa production is seriously threatened by several diseases and pests[5,6], and incorporation of genetic resistance towards these biotic factors is most essential. Further, considering the adverse climatic and edaphic conditions, genotypes tolerant to drought, cold and unfavourable soil are needed. Gearing up banana improvement has chronically remained a challenge, which is several fold difficult than the seasonal crops due to obstacles in conventional breeding such as inherent parthenocarpy, polyploidy, barriers in obtaining viable seeds, long life cycle, etc. Further, the conventional way by mutagenizing vegetative suckers has proved to be futile. Hence banana researchers are left with no option but to adopt 'mutation breeding', particularly in combination with micropropagation and mutagenesis techniques, supported by advanced molecular biological tools.

Embryogenic cell cultures can be utilized with high efficiency than callus or shoot-tip cultures due to several obvious advantages[7]. Understanding their response to the mutagenizing irradiation is then the first step in this direction. The results of our experiments to establish banana cell cultures and subsequently study the effects of gamma-irradiation on their growth are presented here.

Floral apices after the emergence of the female flower were collected from the field-grown plants of banana cv. Grand Naine (AAA). These were surface-cleaned with 70% ethanol and the male flower buds were excised, as described earlier[8]. Cell suspensions were raised by culturing embryogenic calluses in 10 ml of liquid MS medium[9] supplemented with 2,4-D (1 mg/l), biotin (1 mg/l), glutamine (100 mg/l), malt extract (100 mg/l; M_2 medium as described by Cote *et al.*[10]) in 50 ml conical flasks. Actively dividing, densely cytoplasmic embryogenic cells were obtained in 3–4 months. These were regularly subcultured once in ten days and used for irradiation experiments.

Next, 1 ml (~ 50 mg) from the stock cultures was aliquoted into 1.5 ml capacity eppendorf tubes and irradiated at the doses ranging from 0 to 100 Gy (steps of 10 Gy) with gamma rays @ 18 Gy/min in a gamma-220 irradiator. Each of the 11 treatments was replicated thrice. The cells were transferred to 20 ml of the fresh medium of the same composition in 100 ml conical flasks, immediately after irradiation, and maintained on a gyratory shaker (80 rpm) for 40 days and 50% of the spent medium was replaced with a fresh one every ten days. From these, 2 ml cells were plated onto M_2 medium gelled with 0.2% Phytagel (Sigma) for somatic embryo development. All the cultures were incubated under a light intensity of 15 $\mu E\ m^{-2}\ s^{-1}$ at 25 ± 2°C and in a 10/14 h day/night cycle. The fresh and dry cell weights were recorded at a ten-day interval in 1 ml aliquots from each flask. The data were subjected to analysis of variance and the means were graphically represented (Figure 1).

The analysis of variance realized significant treatment mean squares for fresh as well as dry cell weights in each subculture cycle (SC 1 to SC 4; data not presented). The cell cultures irradiated at different doses showed differential growth. Figure 1 depicts the effect of gamma-irradiation on the growth of the cell cul-

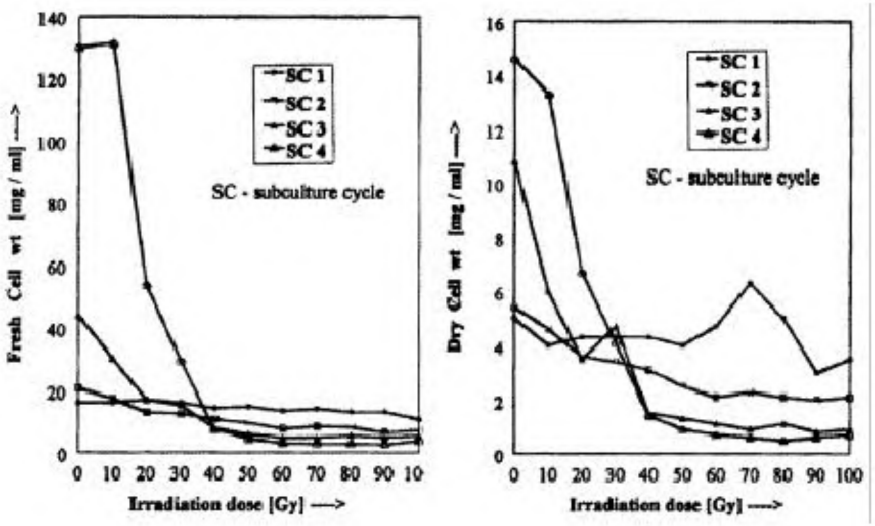

Figure 1. Effect of gamma irradiation on cell suspension cultures of banana cv. Grand Naine.

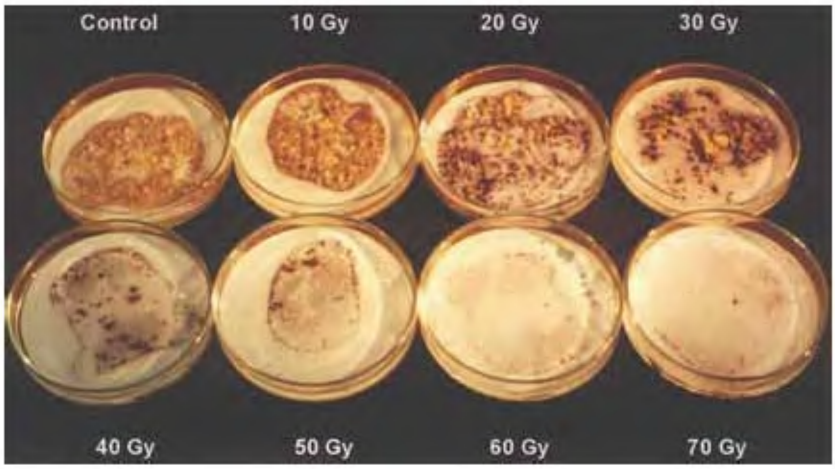

Figure 2. Differentially growing gamma-irradiated cell suspension cultures of banana cv. Grand Naine, eight weeks after planting.

tures, during four subcultures. The growth of cells reduced with an increasing dose up to 30 Gy, and a dose of 40 Gy and beyond was observed to be completely lethal. Cell weight (with each successive subculture) was maximum in control, but reduced with an increasing dose of irradiation. The gain in fresh and dry weights of the cells with increasing irradiation dose was gradual during the first two subculture cycles, but became evident in the third subculture and was maximum in the fourth one. No stimulating effects of the lower doses were noticed. It was interesting to observe that the liquid medium turned dark purple and the degree of darkening was dependent on the dose. No such change in the colour of the medium was found in the case of non-irradiated cell cultures. The plated cells also exhibited similar trend and no cell growth was observed at 40 Gy and above, and somatic embryo development was also not noticed after eight weeks (Figure 2). Preil *et al.*[11] reported that in the cell suspension cultures of *Chrysanthemum morifolium*, the number of regenerated plants decreased with an increase in X-ray dose at low temperatures. Adverse effect of increasing dose of gamma-rays was also observed by Ermolova *et al.*[12] in *Dioscorea deltoidea* cell suspension cultures.

The use of cell cultures for mutagenesis facilitates dealing with large populations under controlled conditions, possibly allowing detection of dominant mutations that occur only at very low frequencies[7], and this can certainly be an added advantage. Especially in the case of banana, the embryogenic cell cultures can be maintained in the liquid medium by regular subculturing, making it a convenient material for genetic manipulation, including induced mutations. Millions of cells can be used for treatment and large populations can be regenerated via somatic embryogenesis for screening. As the somatic embryos develop from single cells, the problem of separating chimeras may not arise and solid dominant mutants can be easily selected. Since banana is a vegetatively propagated crop, improvement through classical methods is difficult. The embryogenic cell cultures and their manipulation using *in vitro* techniques are essential for banana improvement. The procedure reported here for gamma-irradiation and subsequent handling of the cell cultures will be a useful guideline for experiments in this direction.

1. *FAO Production Yearbook*, Food and Agriculture Organization, Rome, 1987, vol. 40.
2. INIBAP, Annual Report, International Network for the Improvement of Banana and Plantain, France, 1992.
3. Sharrock, S. and Frison, E., Networking banana and plantain. INIBAP Annual Report 1998, International Network for the improvement of Banana and Plantain, Montpellier, France, 1999, pp. 42–47.
4. Singhal, V., *Indian Agriculture*, Indian Economic Data Research Centre, New Delhi, 1999, pp. 150–152.
5. Jones, D. R., *Diseases of Banana*, Abaca and Ensete. Cabi, Wallingford, UK, 2000.
6. Chakrabarty, A., Ganapathi, T. R., Mukherjee, P. K. and Bapat, V. A., *Planta*, 2003, **216**, 587–596.
7. vanHarten, A. M., *Mutation Breeding: Theory and Practical Applications*, Cambridge University Press, 1998, p. 353.
8. Ganapathi, T. R., Suprasanna, P., Bapat, V. A., Kulkarni, V. M. and Rao, P. S., *Curr. Sci.*, 1999, **76**, 1228–1231.
9. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
10. Cote, F. X., Domergue, R., Monmarson, S., Schwendiman, J., Teisson, C. and Esclant, J. V., *Physiol. Plant.*, 1996, **97**, 285–290.
11. Preil, W., Huitema, J. B. M. and De Jong, J., *Plant Breed.*, 1991, **107**, 131–134.
12. Ermolova, I. E., Shamina, Z. B. and Butenko, R. G., *Fiziol. Rast.*, 1983, **30**, 974–979.

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