Expression of metallothionein 3-like protein mRNA in sorghum cultivars under chromium (VI) stress

Chromium is used in the leather industry to tan hides. It is not taken up completely by the leather and relatively large quantities of its salts are discharged through solid, liquid and gaseous wastes into the environment. This can have adverse biological and ecological effects. Soil and water ecosystems have been contaminated to an overwhelming extent in the vicinity of the leather industry and this has rendered arable land unproductive and underproductive. There is an urgent need to screen and develop crops suitable for these areas. Metallothioneins (MTs) are cysteine-rich polypeptides encoded by a family of genes. MTs are low molecular weight (6–7 kDa), 60–65 amino acid residue long, cysteine (20 molecules)-rich metal-binding (through mercaptide bonds) proteins[1]. MTs typically contain two metal-binding, cysteine-rich domains that give these metalloproteins a dumb-bell conformation. MT proteins are classified based on the arrangement of Cys residues[2]. Class I MTs contain 20 highly conserved Cys residues based on mammalian MTs and are widespread in vertebrates. MTs without this strict arrangement of cysteines are referred to as Class II MTs and include those from plants and fungi as well as invertebrates. In this MT classification system, phytochelatins are, somewhat confusingly, described as Class III MTs. MT proteins and genes have been found throughout the animal and plant kingdom as well as in the prokaryote *Synechococcus species*. The flood of information about plant genes and cDNAs encoding MT proteins has not been accompanied by a corresponding increase in knowledge about the expression or distribution of MT proteins; this has led to the tendency of researchers to report them as MT-like proteins. Among the various functions attributed to MTs, one important function is conferring tolerance to heavy metals. In plants, a correlation has been observed between MT RNA levels and tolerance to heavy metals in different *Arabidopsis* ecotypes[3], suggesting a role in metal homeostasis in plants. The expression of MT in crops can serve as an important index to select heavy-metal tolerance. In attempting to shed light on their function, investigators have relied primarily on RNA blot hybridization to study the expression of MT genes during development and in response to various environmental factors. More detailed localization of MT mRNAs or MT gene promoter activity has been obtained in a small number of cases through *in situ* hybridization and reporter gene expression studies. RT–PCR has been evaluated as an alternative to Northern blot analysis in quantifying levels of MT mRNA. It was reported[4] that sensitivities of both techniques were similar in detecting induced levels of MT mRNA. These results suggested that RT–PCR may provide a sensitive and quantitative method to evaluate MT gene expression. Many MT genes are expressed at high levels in plant tissues, at least in terms of transcript abundance. The *MT3* gene expression in roots after imposition of 100 µM Cr(VI) for 5 days in 15-day-old seedlings of two sorghum cultivars, viz. K 10 and CO 27 (susceptible and tolerant respectively, based on earlier studies on growth and antioxidant response to Cr(VI)) was studied by enzymatic amplification of RNA by polymerase chain reaction (RT–PCR), as described by Kaplana *et al.*[4]. Crude RNA was extracted from stressed and control plant samples. Selective precipitation of RNA was done to remove DNA contamination by transferring the aqueous phase to a clean 250-ml bottle with 8 M LiCl (1/3 vol) to bring the solution to a final concentration of 2 M LiCl. The mixture was precipitated overnight at 4°C. The extracted RNA was stored in ethanol at – 20°C. RNA recovery was done by centrifugation for 15 min at 10,000 rpm at 4°C and resuspended in l ml water. Next 10 µl was diluted to 1 ml. A260 and A280 was measured, 1 OD260 = 40 µg ml^{-1} RNA. An oligonucleotide primer was co-precipitated with the RNA to maximize the efficiency of their annealing to each other. Following annealing, cDNA was synthesized using reverse transcriptase and enzymatic amplification of this cDNA was then performed by PCR. Amplification of the cDNA by PCR was done by mixing 5 µl cDNA, 5 µl each amplification primer, 4 µl of 5 mM 4dNTP mix, 10 µl amplification buffer and 70.5 µl H_2O. Then 0.5 µl Taq DNA polymerase was added and overlaid with mineral oil. The amplification cycles were as follows: 39 cycles each for 2 min at 55°C, 2 min at 72°C and 1 min at 94°C; 35 cycles each for 2 min at 55°C and 7 min at 72°C. Along with the plant samples, the cDNA of *Arabidopsis thaliana* MT3-like protein gene inserted in plasmid vector pZL1 (Figure 1) was also run as an external control. Gene-specific primers were

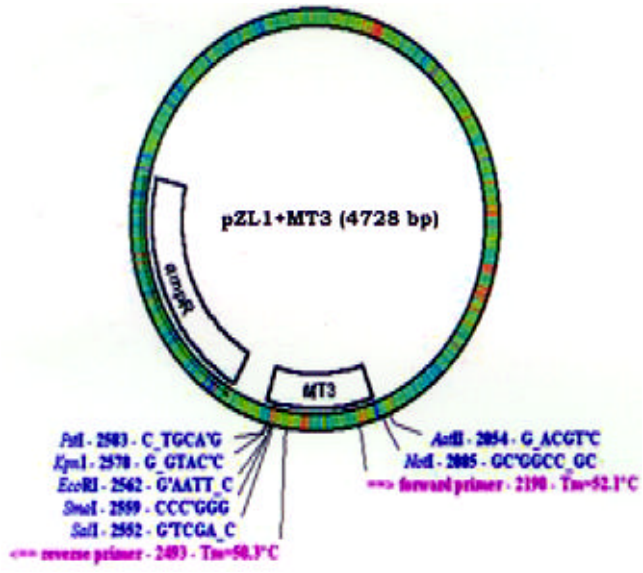

Figure 1. Plasmid vector pZL1 with *Arabidopsis thaliana* metallothionein-like protein (*MT3*) gene cDNA insert.

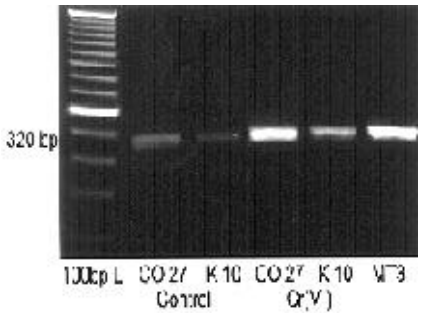

Figure 2. RT–PCR products for *MT3* gene in root tissue of sorghum cultivars K 10 and CO 27. Untreated control are treated with 100 μm Cr(VI) for 5 days in nutrient medium.

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.

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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.