ABSTRACT

Banana (*Musa spp.*) is the fourth most important food crop in the world particularly in India. It is very popular, with high commercial value, has a high demand in markets due to its sweet aroma, taste and increased post harvest life. Banana is used as raw material for manufacture of beverages, fermentable sugars, fragrance, rope, cordage, garlands, shelter, clothing, smoking material and is used in numerous religious ceremonies. However, the shortage of planting material and synchronization of fruit ripening are two major bottlenecks that cause unavoidable trouble to local banana growers. There is a need to establish a micropropagation protocol for this banana cultivar.

Banana is one of the main plants of horticultural interest, which are multiplied by micropropagation, compared with the conventional planting methods. *In vitro* culture techniques of banana plants can produce thousands of plants in a relatively shorter time. Plant tissue culture technique has great potential as a means of vegetative propagation of economically important species. It is important to establish and maintain a virus free stock.

Humic acid is a major component of humic substances, which is the major organic constituent of the soil (humus). Humic acid is a long chain molecule with high molecular weight. It is dark brown and soluble in alkali solution. In soil, humic acid is formed through the chemical and biological humification of plants, animal matter and through biological activities of microorganism. Humic acid is also found in coal and the low grade coal leonardite is particularly the good source of humic acid. Leonardite is an
organic matter and it is the end product of the humification process. The Humic acid is the principal component in the soil which is responsible for transferring nutrients from soil to plants. The humic acid has effect on the plant’s growth that helps in development.

In the present study humic acid was extracted from soft coal leonardite. Alkali extraction was done using different solvent at different concentration. NaOH, KOH and Na₄P₂O₇ were used at 0.1 M and 0.25 M concentrations. The yield of humic acid was reported to be 76.6% when 0.1M KOH was used as extraction solvent and so this concentration of KOH was used for further extraction of humic acid for the present research.

Micropropagation technique was carried out with humic acid along with growth regulators in Murashige and Skoog (MS) medium with different strength (Full, 3/4, 1/2 and 1/4). The sword leaves of the plant were sent to the NRCB (National Research Centre for Banana), Trichy, Tamil Nadu, for virus indexing. The explants that showed negative results are selected for this study. In explants initiation ½ MS + Standard hormones + 0.5 % HA gave highest fresh weight (3.12 ± 1.0 g) and ¼ MS + standard hormones + 0.2 % HA showed highest percentage of bud formation (about 78 %). In explants initiation stage full MS + standard hormones + 0.3 % HA showed highest multiplication rate of about 5.06 ± 0.8 and highest weight of biomass was about 0.796 ± 0.7g. During shooting stage ½ MS + Standard hormones + 0.4 % HA gave the highest (27.0 ± 1.2) number of shoots when compared to all other trails and full MS + Standard hormones + 0.1 % HA gave the highest (10.10 ± 0.3cm) mean shoot length. Humic acid propagated roots were very stronger, denser, more fibrous and giant looking. Humic acid contains
compound which have auxin like activity, predominant results are obtained in rooting stage. $\frac{1}{2}$ MS + Standard hormones + 0.3 % HA gave the highest root length of about 23.1 ± 0.9 cm and $\frac{1}{2}$ MS + Standard hormones + 0.5 % HA gave the highest root mass (416 ± 0.3 mg).

The effects of several concentrations of coal extracted humic acid (CHA) on in vitro propagation of *Musa acuminate* was compared with commercially available humic acids such Keradix humic acid (KHA) and Biochar humic acid (BHA). In this comparative study all the trials were carried out with full strength MS medium. The mean shoot length was higher in CHA when compared to the other samples. The maximum shoot length obtained was observed in plant cultured in MS + 0.1 % HA (4.22 ± 1.17 cm). The root length after the rooting stage was maximum in BHA sample than the other samples. The maximum length obtained was 6.52 ± 1.4 cm at 0.3 % of HA. The plant height was maximum when grown in CHA of 8.35 ± 0.3 cm at 0.2 % of HA.

Antioxidant property had been done for HA exposed plants in order to check for the eventual damage for the plants. Phenol estimation, Superoxide Dismutase, Catalase test, Glutathione S transferase and Ascorbate peroxidase test had been done for humic acid propagated *Musa acuminate* plant leaves. The control native plant had highest activity of 146.2 µg of GAE g/dw followed by Humic Rooting (BHA) having 104 µg of GAE g/dw and Keradix 70 µg of GAE g/dw and Coal of 54 µg of GAE g/dw of polyphenolic activity. Humic Rooting sample (BHA) contained 7 µg/mL of protein but the control plant (i.e. grown in MS media devoid of HA) contained only 1.38 µg/mL of protein. The plant grown with Coal Humic Acid (CHA) reported
2.65 µg/mL of protein. The SOD activity was found to be inhibited highest in plants treated with coal sample of 41.34 % than other samples. It was least in Keradix of 35.03 %. Catalase activity was highest for Humic Rooting of 0.09U/mg catalase and least for commercially available Keradix of 0.021U/mg catalase. The Coal sample exhibited an enzyme activity of 0.048U/mg catalase and control plant exhibited only 0.03U/mg catalase. GST activity was highest in CHA of 582.99 U/mg GST than other three samples and least for BHA of 79.28 U/mg GST. APX specific activity was highest in KHA of 725.31 U/mg APX and least in native plant of 379.38 U/mg APX.

Genomic DNA was isolated from leaf tissue of humic acid propagated *Musa acuminate* plants. The quality and quantity of DNA was checked by agarose gel electrophoresis. The final DNA concentration of each sample was found to be 6.520µg/mL for BHA, 5.982µg/mL for CHA and 6.253µg/mL for KHA.

The genetic fidelity was done with *in vitro* propagated *Musa acuminate* with the use of the three PCR (Polymerase Chain Reaction) based techniques, RAPD (Randomly Amplified Polymorphic DNA), SSR (Simple Sequence Repeat) and ISSR (Inter Simple Sequence Repeat) for the identification of somaclonal variation. Amplified Products were analyzed by using Alphaease Software. There were no polymorphic bands observed in humic acid cultured plants against control plants (Native). So humic acid can be used safely for the commercial production of disease free and genetically stable *Musa acuminate* plants.