



A Biological Approach to Harden the Micropropagated Plants using the Soil Microorganisms- Micro and Macro Nutrient Analysis

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Abstract:

Micropropagation is the technique for rapid multiplication of the selected plants within a short time period. The plants which have been raised in-vitro are delicate and cannot withstand the adverse conditions outside the laboratory ^[1]. Such plants not only face stress above the soil, but also within the soil i.e. in its roots. The roots interact with the soil microorganisms which are beneficial for the plants in performing lots of functions like fixing of nitrogen, etc. However, when these micropropagules grown under sterile conditions are exposed to the field conditions they have reduced immunity to the soil microorganisms, increasing the plant mortality ^[2]. The transfer of micropropagules to the field conditions and the acclimatization stage is the most important step to increase survival of the plants. This has led to an increased focus on environmental conditions during acclimatization such as light intensity, altering CO₂ and sugar concentrations. Another biological approach to help plants acclimatize is to inoculate the plants with suitable microorganisms ^[3]. The present work involves in-vitro raised plantlets of *Musa paradisiaca* which were treated with soil microorganisms during the ex-vitro transfer to check for plant acclimatization and analyze macro-nutrient as well as micro-nutrient composition. The bio-hardened plants showed improved growth in root and shoot length, increased inter node diameter area and number of leaf primordials and also showed an improved drought resistance than controls. Fidelity test was conducted for the treated plants so as to check for the changes in the genetic material due to these alterations. The micropropagated plants have better macro- and micro-nutrient composition but similar genetic content when compared to the control plants proved by the fidelity test.

Keywords: *Micropropagation, Plant acclimatization, Micronutrients, Macronutrients*

Introduction:

It has been over a century since the first attempt at plant tissue culture by Haberlandt in 1902. Since then, several methods have been developed to produce plant tissues in-vitro. These techniques have become the popular means of plant vegetative propagation ^[4, 5]. Micropropagation or clonal propagation in-vitro, is one such method that has been exploited commercially. It is the method of propagating plants from callus, plant organ, a cell or protoplast cultures in sterile conditions ^[6]. The plants that are vegetatively derived from other plants are just clones of the parent plant and have similar genetic content. Thus, micropropagation is the multiplication of individuals that are genetically identical to the parent plant. The advantage of micropropagation over other methods of tissue culture is the reduction in time for the growth of new individuals. The micropropagules thus cultured in-vitro, must then be transferred to the green house or exposed to field conditions. However during this transfer, the mortality rate among the micropropagules is very high as the plants now face various stress conditions such as higher intensity of light, lower humidity levels and soil microorganisms when compared to the sterile in vitro conditions ^[7]. The success of micropropagation is dependent on increasing the survival rate of these cultured plants during the transfer, at minimum costs. To achieve this, the plants must undergo an acclimatization process which enables the plants to deal with this stress in field conditions. The acclimatization process helps the plant compete with the soil microorganisms and to deal with environmental conditions ^[8]. The mortality of plants may be due to the in-vitro culture conditions which alter the morphology, physiology and anatomy of the cultured plants which results in non-functional stomata, poorly developed cuticle and weak root system ^[9]. But it has been proved that treating the plants with certain symbiotic soil microorganisms help the plant in better absorption of nutrients from the surrounding environment, improves the drought resistance and disease

resistance of the plant thereby increasing the survival rate of propagated plants.

The aim of the current research paper is to culture plantlets of *Musa paradisiaca* and use a consortium of soil microorganisms to check the concentrations at which these symbionts help to achieve maximum growth and survival of the plantlets. We have also conducted a macronutrient and micronutrient analysis of these plantlets to determine the physiological status of the plants. A fidelity test has also been carried out to compare the genetic material of the cultured plants with the parent plant material

Material and Methods:

Bio Hardening process

The normal treatment given to the plantlets during the hardening process is as under:

Plantlets are first transferred to distilled water to avoid dehydration and rinsed for a few times, then sprayed with 0.5% w/v benolate fungicide and transferred to the plastic pots filled with sterile peat moss a vermiculite, (1:1 v/v) or the plantlets are grown in a soilless 1:2:1 mixture of sand, peat and perlite containing nitrogen: phosphorus: potassium 18:6:12 with the addition of water as and when required. The whole set is placed under mist conditions with a relative humidity of 90% or above, and a slow increase in ambient temperature to 25°C for onset of growth. The plants and the container are enclosed in interlocking clear polystyrene tumblers- to maintain high humidity. Fungal growth is prevented by spraying applications of benolate every week and pots irrigated with ¼ strength Hoagland solutions for a month. They are then incubated under high light intensity for a 16hr photo period of 25-28°C for two weeks.

Later the pots are transferred to a greenhouse which has shade and humidity, after acclimatization, plants are transferred to potted soil. This was the normal procedure followed by the AG Biotech lab for the hardening process. The above procedure of the hardening was not given to the plants, which were biologically hardened i.e. the plants were not introduced in the fungicide solution, as soon as the plantlets were removed from the MS media they were washed and were incubated with different types of microbial cultures made with different types of concentrations, and combinations, i.e. The microbes were taken in dual concentrations, three, four, and the entire five together (consortium).

The six bacteria that were used to harden the micro propagated plants are:

Vesicular Arbuscular Mycorrhiza, Rhizobia, *Pseudomonas aeruginosa*, *Azospirillum*, Phosphorous Solubilizing Bacteria (PSB) and *Acetobacteria*

Three categories of plants were taken such as the Control, the Ag Biotech lab treated plants and the Biohardened plants.

The micro-organisms were dissolved in distilled water and were treated to the plants, in various combinations, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, the 2.5% of consortium showed the maximum growth of the plants and this parameter was taken as the optimum. The plantlets root length, shoot length, leaf primordial and the Number of leaves were observed.

Plant collection:

The four different samples of plants Control (T1), Plants treated with the micro-organisms, (consortium) (T2), AG biotech treated plants (T3), Ag Biotech plants treated with the consortium (T4) of *Musa paradisiaca* were taken.

Analysis of the plant Material for the Elemental content.

The macro and micro elements were determined using Perkin-Elmer Analyst 300 single beam Atomic Absorption Spectrophotometry and the data was obtained in parts per million (ppm) which was then converted to mg/g. The preparation and extraction of macro and micro elements in the laboratory and determination was used as outlined by Radojevic and Bashkin^[10] for plant samples.

Ashing, Digestion and Analysis of samples

The leaf material is first taken and washed thoroughly with distilled water. The midrib of each leaf is then cut off and only the leafy part is cut into small pieces. The leaf samples were air-dried and were pulverized manually using mortar and pestle. 5.0g of each sample was independently packed into an acid-wash porcelain crucible and then placed in an oven for two (2) hours at 60°C. The crucibles were removed from the oven and cooled. One gram of the leaf samples were taken, to the sample 10ml of tri acid (H₂SO₄, HClO₄, and HNO₃ in the ratio 1:2:4) was added and then kept for digestion overnight. The next day, the samples were kept in the digestion wood chamber and the machine was run for an hour. The heater was then switched off

and exhaust was left on for 30 minutes after which the machine was completely switched off. The digested samples were cooled and filtered through a Whatman No.1 filter paper into a 100ml volumetric flask and then made up to the mark with distilled water. About 1.0g of the grounded leaves was exhaustively processed for various parameters according to the Association of Official Analytical Chemists methods^[11, 12].

The values of the macro and micro nutrients were analysed using the **smart spectrophotometer**

Results and discussion:

The Mean concentration of macro nutrients is in the order of (highest to lowest): **K** (1.74) > **N** (1.43) > **P** (0.09) was recorded.

The table-1 shows that the nitrogen content was less in the plants which were treated with the Consortium and slightly lower with a difference of 0.43% in the plants from the AG Biotech laboratory treated plants along with the consortium, Phosphorous content is less with a difference of 0.16%, and potassium with 1.03%.

The Mean concentration of micro nutrients is in the order of (highest to lowest): **Zn** (6.21) > **Mn** (1.463) > **Fe** (0.96) > **Ca** (0.391) > **Mg** (0.08) as shown in the table 2

Table-1 showing the Macro Nutrient content of the Plants

Plants	N%	P%	K%
T1	1.43	0.09	1.74
T2	2.34	0.11	2.33
T3	1.89	0.16	1.88
T4	2.76	0.27	3.36

Table-2 showing the Micro Nutrient content of the Plants

Plants	Mn%	Ca%	Zn%	Mg%	Fe%
T1	1.463	0.391	6.21	0.08	0.96
T2	1.789	0.14	9.27	0.16	1.43
T3	1.532	0.571	10.07	0.21	1.061
T4	2.035	0.768	14.8	0.32	1.721

The table-1 shows the Manganese content which was less in the plants which were treated with the Consortium and slightly lower with a difference of 0.246% in the plants which were from the AG Biotech laboratory

Treated plants along with the consortium, Calcium content are less with a difference of 0.628%, Zinc with 5.53%, Magnesium with 0.16% and Iron with 0.291%.

The foliar macro and macro nutrient content which was organically grown in Canary Island^[13] where within the optimum ranges and it is less when compared to the plants which were hardened using microorganisms

Fig -1: Graphical representation to compare the Macro Nutrient content among the Four group of plants

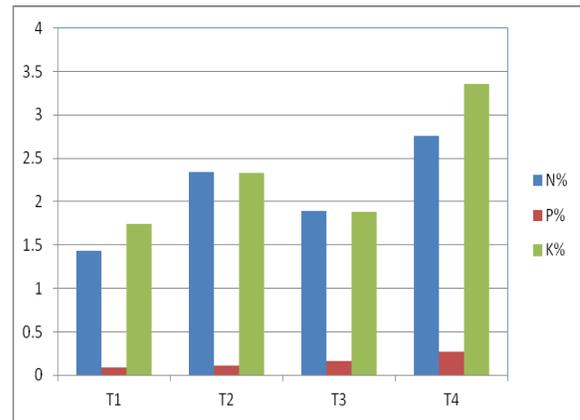
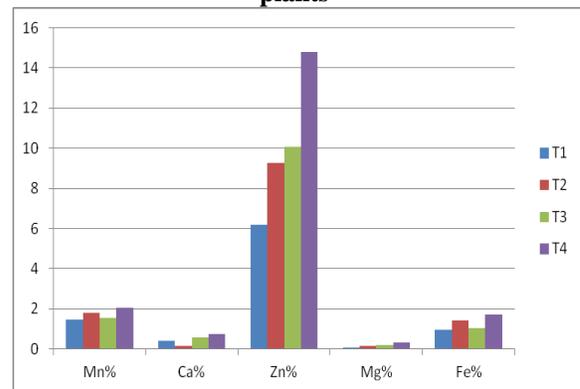


Fig -2: Graphical representation to compare the Micro Nutrient content among the Four group of plants



Conclusion:

In conclusion we established a suitable composition of the soil bacteria in G.naine variety of banana through the hardening process. Our results indicate that the biologically hardened plants showed more growth than the control and the tissue cultured laboratory (AG Biotech), it was observed that the biologically treated plants had high amount of Macro and Micro nutrients which infers that the growth of the plant would be more and the yield of the crop also increases.

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