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Micro Propagation and Plant Strengthening of Tissue Cultured Plants, Inoculated with Several Bacterial Strains

Agriculture

KEYWORDS : Micro-propagation, Bio-hardening, plant acclimatization, VAM, PSB.

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ABSTRACT

Rapid plant production has become a crucial factor in agriculture and horticulture. Most plants tend to lose their vitality due to various growth inhibitors. Micro propagation involves expeditious growth of large number of plants using tissue culture. During this process, In vitro propagation of plants is often associated with high mortality rate during ex vitro establishment phase. Plants when grown in vitro are provided with ideal conditions necessary for their growth, these plants do not have fully functional dermal coverings, they are very fragile, hence, are highly susceptible to growth depreciation when grown outside the lab. Thus hardening and acclimatization of in vitro grown plantlets is critical but necessary phase in micro propagation. When these plantlets are removed from the culture vessel, and exposed to the environment, they suffer high environmental stress, as the soil rhizosphere has several root inhabiting microbes and are known for their endophytic benefits, they reduce the environmental stress, but the In vitro raised plants were never exposed to the soil rhizosphere which in turn may turn be harmful to the root which would be suddenly invaded by the microorganisms.

In this review you'll see that different bacteria have been used to acclimatize the tissue cultured plants with the soil microorganisms which in turn showed enhanced growth of the plants. Different soil samples were collected from various places, from which the six microorganisms were isolated, each one having its unique biological function. The plants were treated with Single parameter of six different bacteria's v.i.z Rhizobium, VAM, Pseudomonas, PSB, Acetobacter and Azospirillum, in different proportions; they were inoculated onto the roots of the plantlets. The bacteria enter into a symbiotic relationship with the roots of these plants. Such bacterial induction helps the plant attain the necessary conditions for uninterrupted growth. The micro propagated plantlets were inoculated by these bacteria during the hardening process. The growth rate caused by each of the bacteria was observed and compared. Striking success in the terms of increment in root length, shoot length, leaf primordial and number of leaves was noted. It was seen that Rhizobium inoculated plantlets showed high frequency of growth and are proven to be the most profitable.

Introduction:

Micro propagation is one of the extensive plant production techniques which involve expeditious growth of large number of plants using tissue culture. During this process, In vitro propagation of plants is often associated with high mortality rate during ex vitro establishment phase [1]. It is globally recognized that tissue culture technique generates homogeneous population of plants endowed with toti-potency of elite mother plants, which are not only agro-climatically adapted but also attributed with vibrant growth, pest resistance and consecutively higher pest resistance [2]. The selected plant is cut into small fragments and cultured. The culture conditions are tuned so that the cells grow into a callus, a mass of cells which look like a small mould. The conditions are then switched so that the callus starts to develop into a small plant "embryo". Once this embryo has grown sufficiently it can be planted out as a plant. The plants that are derived from other plants are just clones of the parent plant and have a similar genetic content [3,4]. The advantage of micro propagation is that it is a time conserving method for a humongous growth of genetically identical plants. The disadvantage of which being; it is skill intensive, and hence much more expensive than conventional breeding, and that it can be only done on plants for which the culture conditions have been worked out. So it is mostly used for propagating valuable, slow growing plants[3]

As stated earlier, during the ex vitro establishment phase the plants require microbial inoculation to help them survive the environmental stress. Those plants that are not exposed to suitable conditions that are provided by these microbes undergo a huge loss mainly due to modified physiology. Plants when grown in vitro are provided with ideal conditions necessary for their growth, these plants do not have fully functional dermal coverings, they are very fragile, hence, are highly susceptible to growth depreciation when grown outside the lab. Thus hardening and acclimatization of in vitro grown plantlets is critical but necessary phase in micro propagation[1]. It has become evident that the colonisation of microbes impacts plant growth and development (bioregulation), plant nutrition (biofertilisation) and plant tolerance by inducing systematic resistance to abiotic and biotic stress (bioprotection)[5]. However, most of the R&D efforts being channelled through companies producing tissue cultured plantlets, information has not come into print being guarded their

technological secrets. Scanty information is, therefore, available regarding national and international scenario of their secondary hardening[6.7]. Problems of growth phase, primary hardening and secondary hardening need to be overcome for arriving at a commercially successful protocol of hardening.[8] The acclimatization process is such one such effort which helps the plant compete with the soil microorganisms and to deal with environmental conditions.[9] The different bacteria induced morphological and physiological changes according to their individual endophytic nature. Rhizobium: Symbiotic bacteria belonging to the genus Rhizobium play an important role in nutrition of leguminous plants by fixing atmospheric nitrogen in the root nodules [10], similarly Acetobacter, Azospirullum help in nitrogen fixation in cereal plants. Pseudomonas, PSB and VAM: these are opportunistic pathogens that help in phosphate stabilization by mineralization of organic phosphorous in soil. The spin centre of this review is to investigate the effects of symbiotic interaction between the microbes and the tissue cultured plants, these bacteria were isolated from their natural or anthropogenic ecosystem using different media, for the identification of these micro organisms gram staining was done , IMVIC test was performed to justify the character and the purity of the organism. These organisms were taken on different proportions (0.5, 1. 1.5, 2, 2.5, 3) and were inoculated in the roots of tissue cultured plants. The concentration at which these microbes helped to attain effective growth and survival rate was compared

Material and Methods:

0.1 **Soil sample collection:**

The different soil sources are:

Sample I: This soil sample was collected from the Paddy field at Manoharabad Rangareddy District, AP

Sample II: This soil sample was collected from the Vegetable field at Anakapally Vizag District, AP

Sample III: The soil sample was collected from Ground nut field at Nandyal,Kurnool District, AP

Sample IV: This soil sample collected from the Pearl Millet field at Sanga reddy, Medak District, AP

Sample V: This soil sample was collected from the Red gram field at Eluru East godavari District, AP

Sample VI: The sample was collected from Sun flower field at Miriyala guda,Nalgonda District, AP

Sample VII: This soil sample collected from the Sea same field at Aurmoor Nizamabad District, AP

Sample VIII: This soil sample collected from the dump site near Hussain Sagar Lake, Hyderabad District, AP.

Sample IX: This soil sample collected from the sea coast of Vizag. Vishakhapatnam District, AP

All the samples were collected in sterile screw capped tubes and care was taken see that the source had different soil characteristics and was from diverse geographical locations.

Screening of micro-organisms

1.2 Serial dilution method:
About 1 gm of each of the above samples was taken into separate conical flasks each containing 100 ml of sterile water. The suspension was kept on rotary shaker for 30 min and kept aside to settle the suspending matter. One ml of the supernatant was serially diluted with sterile water. One ml each, of 10-5 dilution was added to 20 ml of sterile modified differential media, for bacterial screening.

RESULTS:
Gram staining and IMVIC test:

TEST	Rhizobium	Pseudomonas	PSB	Acetobacter	Azospirillum
Gram's staining	-ve	-ve	+ve	-ve	-ve
Methyl Red	+ve	-ve	+ve	+ve	+ve
Voges-proskaeur	-ve	-ve	+ve	-ve	+ve
Indole	-ve	-ve	-ve	-ve	+ve
Citrate	-ve	+ve	+ve	+ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve
Oxidase	+ve	+ve	+ve	+ve	+ve

Each bacterium showed varied colony forming units, the actual number of CFU were analyzed for the inoculation considering the volume of loop to be 0.01ml.

Control-T0- No microbial treatment was given.

T1-innoculated with Rhizobium(2.41x104 CFU/ml),

T2-innoculated with VAM 10gm/plant 50 spores per gram,T3 innoculated with pseudomonas(1.42x103CFU/ml),T4-innoculated with PSB(1.5x106CFU/ml), T5-innoculated with Azospirillum(1.82x105CFU/ml), T6-innoculated with Azotobacter(2.0x107CFU/ml).

Inoculation:
The bacteria were diluted and taken in concentrations of 0.5-3.0% in sterile water, now the tissue cultured plantlets when removed from the agar, washed and then treated by the bacteria, and planted in the sterile soil pit and observed for the growth parameters.

Growth parameters:
Every week, for three months, the length of root, shoot, no of leaf primordial and no of leaves were recorded.

*Control had been allowed to grow independently without microbial interference, for a test investigation to correlate the values of experimental plants. The average of the recorded values has been given below:

Root Length

T0	Conc.	T1	T2	T3	T4	T5	T6
8.2	0.5	9.9	9.1	8.8	5.9	3.8	9.4
7.8	1	8.7	8.9	6.8	11.1	5.2	4.5
9	1.5	10.2	9.8	5.5	7.9	4.6	4.9
6.2	2	9.8	7.8	9.4	7.3	4.3	11.8
8	2.5	14.4	7.8	9.4	7.3	4.3	11.8
2	3	5.6	3.6	2.4	3.8	1.2	5.3

Shoot Length

T0	Conc.	T1	T2	T3		T4	T5	T6
10.5	0.5	15.6	15.6	16.2		14.2	14.4	14.3
11.5	1	19.7	15.3	14.2		18.4	15.6	11.5
14	1.5	18.2	16.5	13.8		10.1	11.9	9.9
12.2	2	18.2	16.5	13.8		10.1	11.9	9.9
14	2.5	22.5	9.9	17.2		18.3	15.9	16.9
5	3	6.2	3.5	4.2		5	3.6	3.9

Leaf primordial

T0	Conc.	T1	T2	T3	T4		T5	T6
0	0.5	0	0	0	0		0	0
0	1	1	0	1	1		0	0
0	1.5	1	1	1	1		0	0
0	2	1	1	1	1		0	0
1	2.5	1	0	0	0		1	1
0	3	0	0	0	0		1	0

No. Of Leaves

T0	Conc.	T1	T2	T3	T4		T5	T6
4	0.5	6	5	3	3	4	4	0.5
5.2	1	7	4	5	4	2	5.2	1
4.4	1.5	8	6	4	4	5	4.4	1.5
6.2	2	8	6	4	4	5	6.2	2
6.9	2.5	9	4	6	5	6	6.9	2.5
2	3	5	2	2	3	2	2	3

It was seen that the root length was maximum (14.7) in the parameter of 1.5 in T1 (inoculated with rhizobium). The shoot length showed an optimum value of (19.7) in the parameter of 1.0 in T1(inoculated with rhizobium), T4(inoculated with PSB) showed a much closer shoot length to the optimum value (19.1) in the parameter of 1.0. The numbers of leaves were found to be maximum in roots inoculated by rhiobium (8 leaves in the parameter of 1.5). The leaf primordial showed more or less an equal value in all the inoculated plants. All the readings in the experimental plants that were treated with micro-organisms showed a prominently satisfying result as compared to the control plant T0.

Survival rate: After one week of inoculation of the soil micro-organisms to the micropropagated plants, the plantlets were showing a good growth in all the concentrations, but when the concentrations of inoculums (micro-organisms) increased from 3% and beyond, the plantlets showed high mortality rate.

Discussion: The results obtained were *ANALYZED AND* paralleled to various other conclusions, regarding, more or less the same type of investigation. Inoculation of plantlets with micro symbionts has been recommended for reducing the stress of acclimatization, providing faster growth and better establishment of micro-propagated plants.[11] To begin with, most of the experimentalists saw that all the treatments with biofertilizers (T1 to T6) were significantly better over the control (T0). This was logical in view of complimentary functions of the various bacteria as biofertilizers. [12] Plant growth regulators can induce the formation of hyperhydricity in tissue cultures of several species (Ziv, 1991, Fraguas et al., 2004, Toth et al., 2004, Fragaeus et al., 2009). Many reports now indicate that the transfer of tissue culture raised plantlets to field conditions after biological hardening with micro-organisms showed a good growth and high survival rate and better growth performance. [11, 13, 14, 15,]. The root length, unlike other parameters no of leaves and shoot length, showed an insignificant increase in mycorrhizal plants as against the control in micropropagated banana plants. [16]Similar experiments involving the use of various tissue cultured plants bio hardened with different micro-organisms, have resulted to be profitable in the terms of symbiotic effects on plant growth. The plant Tylophora indica was grown using many potting mixtures of soil, vermicompostand biofertilizer-Azotobacter & pseudomonas in different combinations showed the highest survival rate[15] Respiration rates were significantly reduced while photosynthetic rates were enhanced up to two times in AMF treated plants. The foliar tissues in treated plants showed improved nutrient contents especially for the P, Mg, Zn and Mn. Among the AM strains, G. mossae showed good (42.5%) root colonization for Pusa Urvashi, while G. manihotis was most responsive for Pusa Navrang. Though some of the strains did not give good colonization but drastically reduced the microbial attack. High plantlet survival was noted for the mycorrhizal plants after glasshouse and field transfer [16].

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