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Biotization and Enhanced Growth of Tissue Cultured Plants by Inducing Dual Combination of Soil Bacteria

KEYWORDS: PGPR, VAM, PSB, acclimatization

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ABSTRACT *Tissue culture has paved way for the clonal multiplication rapidly of elite plants, crop improvement, genetic transformation, basic morphogenesis studies, and conservation and exchange of germplasms. The plantlets usually exhibit high mortality rate upon their transfer to soil as a result of transplantation shock caused by abiotic and biotic stress and weak root system in the absence of beneficial microflora. The role of such different microbes has been enumerated in our study. Soil microbes where obtained from different soil beds, each varying in their biological activity. A single bacterium was checked for their role in plant growth then dual parameters of six different microbes, namely; Rhizobium, Azotobacter, Azospirillium, Psuedomonas, VAM and PSB were used in different proportions. Root induction of such microbes to the micropropagated plants showed increase in the root length, the shoot length, leaf primordia and the number of leaves, fresh and dry weight was more, when compared to the control and single bacteria used. This present study checks the plant growth in dual bacterial treatment given to the plants. Later there was a fedility test conducted to the biotized plants to prove the plants genetic material was of true type.*

INTRODUCTION:

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods, this is an attractive and alternative method for true to type[1]. Rapid and mass multiplication of planting materials under disease free conditions is used to multiply novel plants, such as those that have been genetically modified or bred through conventional plant breeding methods. Micropropagation has its own drawbacks of hardening, these plants when transferred to the soil exhibit high mortality rates [2]. *THE ULTIMATE SUCCESS OF MICROPROPAGATION ON A COMMERCIAL SCALE DEPENDS ON THE ABILITY TO TRANSFER PLANTS OUT OF CULTURE ON A LARGE SCALE, AT LOW COST AND WITH HIGH SURVIVAL RATES [3].* The plants *EXHIBIT HIGH MORTALITY RATE UPON THEIR TRANSFER TO SOIL AS A RESULT OF TRANSPLANTATION SHOCK CAUSED BY ABIOTIC AND BIOTIC STRESS AND WEAK ROOT SYSTEM IN THE ABSENCE OF BENEFICIAL MICROFLORA[4].* Even 5% mortality rate causes a huge loss during commercial plant production. The glass house and field posses relatively lower humidity, higher light intensity, and septic environment that are stressful to micropropagated plants as compared to invitro conditions [5]. The process of adapting a plant to the location where it will eventually be grown is called "hardening-off" and a failure to harden-off plants is the leading cause of stunted growth or death (Mary Finn) after transplanting to the garden, normally we use a weak fertilizer solution to get transplants growing again and help avoid transplant shock. Several methods have been tried for the hardening of the tissue culture raised plants for successful field establishment (Bhojwani and Dhawan in 1989, Bisht et al.1998). However, the acclimatization procedures adapted on the tissue culture raised plants have not been very satisfactory in providing quality transplants for the field (Ziv in 1995). Apart from endophytic fungi some soil bacteria also promote plant growth and such beneficial bacteria are referred to as plant growth promoting rhizobacter (PGPR)*[6]. Chemical transformations like degradation of organic matter, disease suppression and nutrient transformations inside roots are performed by soil bacteria. Various studies revealed the recent trends, progress and development with respect to mineral phosphate solublization by various plant growths promoting rhizobacteria[7]. Solubilization of phosphates and Phosphorus is considered as the fundamental macronutrient after N which have significant role for improved plant growth and yield (Podile and Kishore 2006, Ali et al., 2012).*

The purpose of this work was therefore to evaluate the role of microorganisms present in the soil in enhancing the mortality and growth rate of micropropagated plants, we have isolated various soil bacteria by using different methods like screening and staining from natural resources in various parts of Andhra Pradesh, We have inoculated the micropropagated roots of MUSA PARADISIACA with single bacteria. Plants induced with the bacteria RHIZOBIUM showed more root length, shoot length, leaf primordia and number of leaves. Advancing the experiment we used dual combinations of PGPR, i.e RHIZOBIUM, AZOTOBACTER, AZOSPIRILLUM, PSEUDOMONAS, PSB & VAM.

Material and methods:

Isolation and screening and of microorganisms: There were seven different soil samples collected from various districts of Andhra Pradesh and the microorganisms were isolated using serial dilution method and documented.

Screening of Microorganisms: There were screening tests performed for each bacteria like growing the microorganisms in differential media, IMVIC tests were performed, staining tests were conducted and documented[8].

Enumeration of Bacteria: The bacteria which was isolated was enumerated, and the cfu for each particular bacteria was done.

Inoculation: The six bacteria which were isolated from the soil samples were inoculated to the micropropagated plants using single bacteria as a parameter and compared against control, the results were document[8]

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In continuation of this work, the PGPR was given to the micropropagated plants in dual combinations there were 15 combinations made, i.e T1- Rhizobium & VAM (2.41X104 CFU/ML,10GM/PLANT 50 SPORES PER GRAM), T2- Rhizobium & Azotobacter (2.41X104 CFU/ML, 2.0X107CFU/ML), T3- Rhizobium & Azospirillum (2.41X104 CFU/ML, 1.82X105CFU/ML), T4- Rhizobium & Pseudomonas (2.41X104 CFU/ML, 1.42X103CFU/ML), T5- Rhizobium & PSB, (2.41X104 CFU/ML, 1.5X106 CFU/ML),T6- Azotobacter & Pseudomonas (2.0X107CFU/ML, 1.42X103CFU/ML), T7-Azotobacter & Azospirllum (2.0X107CFU/ML, 1.82X105CFU/ML) , T8- Azotobacter & PSB (2.0X107CFU/ML, 1.5X106 CFU/ML), T9- Azotobacter & VAM, (2.0X107CFU/ML, 10GM/PLANT 50 SPORES PER GRAM), T10- Azospirillum & PSB (1.82X105CFU/ML, 1.5X106 CFU/ML), T11- Azospirillum & Pseudomonas (1.82X105CFU/ML, 1.42X103CFU/ML) , T12-Azospirillum & VAM (1.82X105CFU/ML, 10GM/PLANT 50 SPORES PER GRAM), T13-Pseudomonas & VAM (1.42X103CFU/ML, 10GM/PLANT 50 SPORES PER GRAM), T14- Pseudomonas & PSB, (1.42X103CFU/ML, 1.5X106CFU/ML) , T15- VAM & PSB (10GM/PLANT 50 SPORES PER GRAM, 1.5X106CFU/ML). T0-Control was not treated with any microorganisms.

These combinations were inoculated to the roots of the micropropagated plants and the results were compared against control.

All the above 15 combinations were inoculated to the roots of the micropropagated plants in various concentrations of 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, for each combination and parameter 10 plants were taken, the root length, shoot length, leaf primordia and no of leaves were recorded every week and it was continued for 12 weeks the result values were compared against the control. The 2.5% concentration had showed maximum growth so this parameter was taken as optimum concentration.

Nutrient analysis: The micro and macro nutrient analysis was done by Perkin- Elmer Analyst 300 single beam atomic spectrometry, the plants which were treated with PGPR showed enhanced nutrient content in it and documented[9]

Genetic stability test: As there were increased levels of nutrients, proteins and growth of the micropropagated plants inoculated with PGPR was observed, Fedility test was done to confirm the genetic stability of these plants and it is doucumented[10].

Results and discussion:

Enumeration of Bacteria: EACH BACTERIUM SHOWED VARIED COLONY FORMING UNITS, THE ACTUAL NUMBER OF CFU WAS ANALYZED FOR THE INOCULATION CONSIDERING THE VOLUME OF LOOP TO BE 0.01ML.

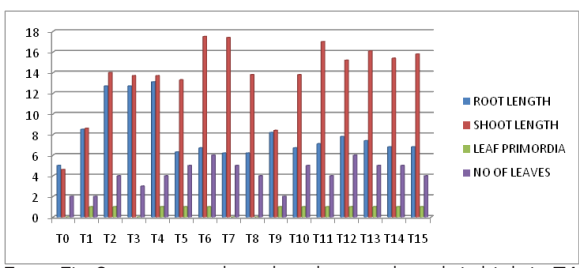
THE VIABLE COUNTS RANGED IN THE SOIL SAMPLES WERE RHIZOBIUM(2.41X104CFU/ML), PSEUDOMONAS (1.42X103CFU/ML), PSB (1.5X106CFU/ML), AZOSPIRILLUM (1.82X105CFU/ML), ACETOBACTER(2.0X107CFU/ML), VAM (10GM/PLANT 50 SPORES PER GRAM)

SURVIVAL RATE: AFTER ONE WEEK OF INOCULATION OF THE SOIL MICROORGANISMS 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

TABLE:1

TEST SAMPLE	ROOT LENGTH	SHOOT LENGTH	NO OF LEAVES	LEAF PRIMORDIA
T0	5.0	4.6	0	2
T1	8.5	8.6	1	2
T2	12.7	14	1	4
T3	12.7	13.7	0	3
T4	13.1	13.7	1	4
T5	6.3	13.3	1	5
T6	6.7	17.5	1	6
T7	6.2	17.4	0	5
T8	6.2	13.8	0	4
T9	8.2	8.4	1	2
T10	6.7	13.8	1	5
T11	7.1	17	1	4
T12	7.8	15.2	1	6
T13	7.4	16.1	1	5
T14	6.8	15.4	1	5
T15	6.8	15.8	1	4

Figure: 2



From Fig:2 we can analyse that the root length is high in T4 >T2,T3>T1, Shoot length is high in T6>T7>T13, No of leaves is high in T6 & T12, Leaf primordia is almost similar like other ones

Conclusion:

A search to the other avenue of biofertilizers with multipurpose activities main emphasis to the plant productivity has been increased to increase the soil health. Azotobacter have been universally accepted as a major inoculum used in biofertilizer to restore the nitrogen level into cultivated field [11].The ability of Azospirillum to fix the atmospheric nitrogen in to the soil and make it available to the plants is an important trait in sustainable farming for increasing crop yield [12]. VAM fungi increase growth of plants in soil where P deficiency limits the growth of non-mycorrhizal plants [13]. In our study when the micropropagated plants were treated with different dual combinations they showed a good growth when they were inoculated with 2.5% concentration of the PGPR.There was enhanced growth in the root seen which was inoculated with the dual combination of Rhizobium &Pseudomonas, in shoot length it was Azotobacter & Pseudomonas, No of leaves it isAzotobacter & Pseudomonas, The leaf primordial was similar in most of the parameters. As it was being said Azotobacter has been proved to be used as thefertilizer [11]. Frommel et al also reported that Pseudomonas strain PSJN enhanced the tolerance to transplanting stress in potato and was found the most effective plant growth promoting bacterium under invitro conditions [14].

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