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Plant growth promoting microorganisms in micropropagation

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Abstract
Micropropagation is a technique of producing new plants from single cell, tissue or small pieces of vegetative material. Micropropagation may be used in herbaceous plants such as strawberry, gladiolus and woody plants like apple, rose *etc*.
Trichoderma harzianum inoculation in pomegranate cuttings resulted in significant increase in the number and length of primary roots (Satish Kumar, *et al*., 2001).
Azospirillum lipoferum inoculated to cuttings of one year old runner shoots of pepper had increased root formation, root development, root weight and germination of cuttings (Govindan and Chandy 1985).
Inoculation of Arbuscular mycorrhizal fungi in the cashew cuttings increased the plant height, stem girth and total biomass (Lakshmipathy, *et al.,* 2000).
Arbuscular mycorrhizal fungi increased the uptake of P, N, Mg, Cu, Fe, and Zn *etc.* in the micropropagated grape vine plants (Hare Krishna, *et al*., 2006).
Application of *Glomus fasciculatum* in the micropropagated sugar cane plants increased the root biomass, improved survival rate, significant increase in percent colonization, sugar content, cane height and cane yield (Guleri, *et al*., 2005).
Plant growth promoting microorganisms have the potential to contribute significantly to the development of sustainable agricultural system. Cost of chemical growth regulators and hazardous effects on cuttings can be eliminated to a greater extent with the aid of plant growth promoting microorganisms.

Keywords: micropropagation, promoting microorganisms

Introduction
Soil is considered as a store house of microbial activity. Some beneficial microorganisms preferentially associate on roots of crop plants. Plant roots constantly alter the rhizospheric soil environment by secreting root exudates. Converts non available form of nutrients to available form. It helps in uptake of nutrient elements from soil. They are very safe for human beings, animals and environment Plant growth promoting microorganisms application in cuttings improves the root development. Root exudates consists of sugars, organic acids and secondary metabolites. Plants provide the major source of carbon for maintenance of microbial community in the rhizosphere micro flora. More over the plant itself depends on the ability of microbial community to make required nutrients available including Nitrogen, Phosphorus and Iron some soil bacteria preferentially associate with the roots of crop plants and exert beneficial effects on their hosts.

Factors responsible for stimulation of plant growth
1. Ability to produce or change the concentration of the plant hormones like indole acetic acid (IAA), Gibberellic acid, cytokinins and ethylene
2. Nitrogen fixers: some microorganisms like Rhizobium which fixes atmospheric nitrogen
3. Antagonism against phytopathogenic microorganism Solubilisation of mineral phosphates and other nutrients: like Zn, P, Mo, B, S, Cu.

Mechanism of plant growth stimulation
1. Increased availability and uptake of nutrients: Zn, P, Mo
2. Siderophore production: Fe chelating bacteria
3. Production of plant growth promoting substances: indole acetic acid (IAA), Gibberellic acid, cytokinins and ethylene

Siderophore produced by plant associated rhizoshere bacteria

S.N	Siderophore	Bacteria
1	Pyoverdin	*Pseudomonas flourescens* *Pseudomonas putida*
2	Catechols a)Agrobactin b)Enterobactin c)Azotochelin	*Agrobacerium tumifaciens* Enterobaceriaceae family *Azotobacter vinelandii*
3	Other types Rhizobactin Citric acid Azotobactin	*Rhizobium meliloti* *Bradyrhizobium japonicum* *Azotobacter vinelandii*

Important plant growth promoting microorganisms:
Nitrogen fixers

1. Aerobic Symbiotic: ex: *Rhizobium sp.*
   Frankia Asymbiotic: ex: *Azotobacter sp.*
2. Facultative anaerobes ex: *Bacillus sp*
3. Microaerophyllic ex: *Azospirillium sp.*

Importance

1. These organisms secrete growth promoting substances like auxin IAA, Indole-3-acetamide(IAM) which helps to increase root biomass, root branching and root hair development
2. These organisms fixes atmospheric nitrogen
3. Resistant to diseases
4. Increases crop yield

P- solubilisers

Bacillus megaterium, Pseudomonas fluorescence, Aspergillus niger, Thiobacillus ferroxidans, Thiobacillus thioxidans, Trichoderma sp., Streptomyces

Importance

1. These organisms secretes growth promoting substances are produces organic acids auxin, IAA, Indole -3-acetamide which helps to enhance rooting They secretes citric acid, glycolic acid, fumaric acid, oxalic acid, succinic acid
2. Produces siderophores: Fe chelating compounds produced by PSB that chelate iron from iron phosphates and there increases P availability in acid soils
3. Resistant to plant diseases
4. Enhances root biomass and photosynthetic area

P-mobilisers

1. Ectomycorrhiza: Ex: *Pisolithus tinctorus, Boletus sp*.
2. Endomycorrhiza: Ex: *Glomus sp. Scelerocystis sp. Acaulospora sp. Entrophosphora sp. Gigaspora sp. Scutellospora pellucida*
3. Ericoid: Ex: *Pezizella ericae*
4. Orhid: Ex: *Tulasnella sp*.

Importance

Helps in Uptake of nutrients Zn, Mo, Fe, Cu
Helps in uptake of water
Helps to overcome transplantation shock
Protects the plant from root born pathogen
Secretes growth promoting substances
Plants are drought tolerant

Plant growth promoting microorganisms acts as a biocontrol agents

Biocontrol	Crop	Causal organisms/disease
Pseudomonas fluorescens	Cotton Cotton Cotton	Damping off *Rhizoctonia solanii* *Pythium ultimum*
Pseudomonas putida	Radish Beans Potato	Fusarium wilt *Fusarium solanii* *Erwinia carotovora*
Bacillus subtilus	corn	*Fusarium roseum*
Rhizobium and Bradyrhizobium	Soybean Mungbean Sunflower	*Macrophomina phaseolina* *Rhizoctonia solanii* *Fusarium solanii*
Bacillus subtilus	Mungbean	*Meliodogyne javanica*

Role of mycorrhiza in Micropropagationed plants

Studies conducted at the Centro miglioramento genetico e biologia vite, CNR, Grugliasco Micropropagated shoots of kiwi fruit were rooted in media containing a dyeDye is to distinguish *in vitro* from *in vivo* formed roots Transfer to soil, plants were inoculated with VAM fungus *Glomus mosseae* Presence of dye did not prevent root colonization by VAM fungus 4 to 5 weeks after transplanting both *in vitro* and *in vivo* formed roots were colonized by VAM fungus

Role of mycorrhiza in micropropagated sugar cane

Studies conducted at department of botany, Gulburga university Shoot formation was obtained from leaf explants of sugarcane var CO 419 when cultured on MS medium supplemented with 3mg/lit 2-4D and 10% coconut milk to obtain a friable sugarcane callus and further sub cultured on MS medium supplemented with 2mg/lit kinetin and 10%coconut milk for shoot induction. Regenerated shoots were transferred to MS liquid medium. Using the following treatments T1 MS medium supplemented with NAA (5mg/lit) with 10% coconut milk

T2 MS medium supplemented with *Glomus aggregatum* spore extracted (2500spores/lit) and 10% coconut milk

T*3* MS medium supplemented with VAM root extract (10g/lit) and 10% coconut milk

T4 MS medium with non VAM root extract (10g/lit) and 10% coconut milk

Treatments	Root initiation	No. of roots /shoots	Length of roots after 20 days	Length of roots after 40 days
T1	8-12 days	10-15	0.4-0.6 cm	1-2 cm
T2	3-6	3-12	1.5-3	3-4
T3	2-6	30-50	0.5-1	1.5-3
T4	-	-	-	-

Sujan Singh *et al*., 2002

***In vitro* and *in situ* mycorrhization micro propagated sugarcane plants and effects on yield:**

Colonization of *in vitro* grown sugarcane roots by VAM:

Days after infection	%colonization
10	48
20	68
30	78

Guleri *et al*., 2005

Root biomass of *In vitro* sugarcane cultures in a laboratory is well as after one month of transplantation to glass houses:

Treatment	laboratory	Glass house
Control	1.97	34.51
VAM	2.45	70.10

Guleri *et al*., 2005

Survival rate of micropropagated sugarcane plants during transplantation

survival		
Treatment	**Laboratory to glass house**	**Glass house to field**
control	60	80
In vitro VAM infected	90	99
Exvitro VAM infected	86	100

Guleri *et al*., 2005

Arbusular-Mycorrhizal fungi alleviate transplantation shock in micro propagated grapevine

Nutrient changes in grapevine plantlets caused by different arbuscular-mycorrhizal fungi during acclimatization

Treatment	P (%)	N (%)	Mg (%)	Cu (µg/g)	Fe (µg/g)	Zn (µg/g)
Control	0.31	1.19	1.31	6.05	42.26	53.70
Acaulospora laevis	0.68	3.25	2.44	11.34	43.65	120.30
Acaulospora scrobiculata	0.66	2.43	2.47	9.70	53.39	78.23
Entrophospora colombiana	0.33	3.28	3.23	9.97	63.77	81.27
Gigaspora gigantia	0.52	2.16	2.24	7.28	53.32	75.50
Glomus manihotis	0.62	2.96	3.53	10.94	58.83	106.57
Mixed AMF inoculum	0.72	2.83	2.86	12.49	53.99	85.10

Hare Krishna *et al*., 2006

Conclusion

Plant growth promoting microorganisms have the potential to contribute significantly to the development of sustainable agricultural system. Cost of chemical growth regulator and hazardous effects can be eliminated to greater extent with the aid of plant growth promoting microorganisms.

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