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ISOLATION OF RHIZOSPHERIC BACTERIA AND THEIR EFFECT ON GERMINATION OF TOBACCO SEED AND GROWTH OF SEEDLINGS

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ABSTRACT

Rhizosphere bacteria were isolated from root zones of tobacco (*Nicotiana tabacum* L.) plants. They were cultured in laboratory and investigated for their effects on germination and growth of tobacco seedlings. Isolates thrive on root exudates in the rhizosphere and benefit the plants by altering the solubility and availability of mineral nutrients and protecting the roots from invasion of pathogens by creating an antibiotic barrier around the root. Present study revealed that rhizospheric bacteria do not effect the germination of tobacco seeds. Tobacco rhizospheric bacteria were found to produce better growth of tobacco seedlings in pot cultures.

Key words: Bacteria, Rhizosphere, Soil microflora, Tobacco.

INTRODUCTION

Rhizosphere is a zone between the root surface and the soil adjacent to the roots (Clarke, 1949). The bacteria which live in this zone may remain in the soil adhering to the roots. The bacteria inhabiting in the rhizosphere are called rhizobacteria (Hiltner, 1904). They form a protective barrier to the root from infection of parasites (bacteria, fungi, actinomycetes, nematodes etc) (Shamima and Rahman, 2007). Rhizobacteria also excrete antibiotic substances and protect the roots from plant parasites by toxic effects (Subhashini and Padmaja, 2009a). In exchange they get their nutrition from the root exudates (Narula *et al.*, 2009). Thus symbiotic relationship is established between the host plant roots and the rhizosphere bacteria (Starkey, 1938). The rhizobacteria are characterized by their association with specific host plants and their habitat (Vessey, 2003).

Tobacco (*Nicotiana tabacum* L.) nurseries are raised in nutrient deficient sandy soils with heavy chemical fertilization and fungicides. The characterization and utilization of rhizospheric bacteria offers an opportunity to cultivate healthy seed beds with nutrient efficiency. Ethylene-producing rhizosphere bacteria *Pseudomonas* sp. and *Klebsiella oxytoca* control root parasite striga-infestation in maize and sorghum (Babalola *et al.*, 2002).

Population of microflora in rhizospheric soil is higher than the soil without rhizosphere which indicates the influence of living roots in the soil. The present work has been undertaken to isolate and characterize the bacteria from the tobacco field rhizosphere. Fresh cultures of rhizospheric bacteria were inoculated in the sterile soil in pot cultures of young seedlings of tobacco to see the effect of these bacteria on germination of tobacco seeds and their contribution to growth of seedlings.

MATERIALS AND METHODS

Rhizosphere bacteria were isolated from tobacco field rhizosphere and one strain of *Pseudomonas sp.* was obtained from the laboratory of the Department of Plant Pathology, TNAU, Coimbatore. Fresh and dried seeds of tobacco (VT-1158) were used for germination and raising the seedlings. Nutrient agar medium was used for culturing the bacteria in glass-tubes and petri dishes. Both solid and liquid culture media were used in the experiment.

Soil samples were collected from rhizosphere soil of tobacco field. Soil from the established tobacco transplants at 12 cm radius and depth from the surface were collected and sun dried.

Earthern pots of 30 cm diameter were filled with the rhizospheric soils and filter papers were put apressed on the soil and sterile liquid bacterial culture medium was pipetted on the filter paper to allow growth of bacteria in colonies on the filter paper from the soil. The pots were kept at room temperature (25^0C). Well-grown bacterial colonies were picked up with a sterile wire loop and cultured separately in liquid culture tubes. They were numbered numerically. Streaking method was used for growing bacterial cultures in plates.

RESULTS AND DISCUSSION

Tobacco seeds were germinated on water soaked filter paper in sterile culture in petri dishes and 15 day old germinated seeds with plumule and radicle were transplanted in pots containing sterile soil. Germination test was replicated thrice with treatments, native rhizospheric bacterial inoculum of mixed cultures, *Pseudomonas fluorescens* (TNAU-isolate) and control without spray. The results showed 100 per cent germination in all the treatments indicating the effect of imposed treatments. The pot culture experiment was replicated eight times. Sterile sandy loam soil was used to fill the pots which were fertilized as per standard recommended doses. The treatments as mentioned in the germination test were applied as aqueous spray. Uniformly aged tobacco seedlings were harvested after 60 days of transplantation to record observations.

The results indicated that the native rhizospheric bacterial inoculum of mixed cultures applied as spray @10 ml (450 cfu / ml) significantly improved the growth and nutrient status of tobacco seedlings followed by *Pseudomonas* (TNAU). Control without application of bioagents was significantly inferior to the rest of the two treatments.

The photographs (Fig. 1) show that in soil culture the seedling growth is much better with the rhizosphere bacteria in comparison with those in the control and with *Pseudomonas*. This might be due to the symbiotic effect of rhizospheric bacteria with the seedlings (Subhashini and Padmaja, 2010). Rhizospheric bacteria were re-isolated from the rhizosphere soils of pot cultures of young seedlings with the help of sterile wire loop by touching the roots and soil around the roots.

It is assumed that the root exudes of different plants differ considerably in their carbon/ nitrogen ratio (Kumar and Narula, 1999). Leguminous plants, for instance, excrete relatively large amounts of N-containing compounds, particularly amino acids, whereas sugar cane and sugar beets excrete relatively large amount of sugars. If this assumption is correct, it is expected that (a) *Azotobacter* respond to root exudes having a high C/N ratio. When the roots are growing in heavily buffered alkaline soil that (b) other types of soil nitrogen fixers which are not represented by an acid reaction (*Clostridia*, *Azotobacte*r and *Pseudomonas*) are selected in the rhizosphere (Vazquez *et al.*, 2000). Rhizospheric microorganisms produce beneficial effects on plants by fixation of molecular nitrogen by their solubilizing and chelating effects on diverse mineral nutrients. By the extent to which they did on the roots in the absorption and storage of mineral nutrients by their synthesis of accessory growth substances and by their suppression or control of soil borne plant pathogen (Subhashini and Padmaja, 2009b). Data revealed

TABLE 1: Effect of rhizospheric bacteria on growth of FCV tobacco seedlings.

Treatment	No. of leaves	Leaf area (cm^2/plant)	Total chlorophyll (mg/gm)	Dry weight (g)	Total length (cm)	Height of the seedlings (cm)
Control	4.25	160	0.73	2.26	3.61	9.30
Pseudomonas	4.75	191	0.93	4.02	5.12	10.62
Rhizospheric bacteria	5.62	595*	1.28	6.76	5.86	12.16
S Em ±	0.1703	1.8692	0.01	0.2	0.11	0.07
C.D. at 5%	0.5011	5.4985	0.03	0.07	0.34	0.21
CV %	9.88	7.4838	2.69	1.47	6.68	1.89

*Highly significant.

TABLE 2: Effect of rhizospheric bacteria on nutrient content of FCV tobacco seedlings.

Treatment	N (%)	P (%)	K (%)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
Control	2.69	0.18	2.76	59.30	160.75	581	28.84
Pseudomonas	2.79	0.27	3.22	65.94	225.62	943	42.21
Rhizospheric bacteria	2.89	0.31	3.88	72.23	261.62	1020	48.34
S Em ±	0.01	0.004	0.05	0.49	2.60	11.81	0.25
C.D. at 5%	0.04	0.01	0.14	1.46	7.66	34.75	0.73
CV %	1.29	4.89	4.10	2.14	3.41	3.94	1.77

Control With Rhizospheric Bacteria With *Pseudomonas*

FIG 1: Soil culture.

that the growth of tobacco seedlings was enhanced and produced leaves with *Pseudomonas* as well as with other rhizosphere bacterial mixtures compared to the control, indicating beneficial effect of rhizosphere bacteria (Volkmar and Bremer, 1998). Data clearly indicates that the rhizosphere bacterial mixtures are more efficient than the single rhizospheric bacterium *Pseudomonas* alone. Rhizospheric bacteria may, therefore, be used as a substitute for fertilizer, they produce CO_2 around the roots by respiration and this in turn is converted to carbonic acid with water ($CO_2 + H_2O = H_2C_O3$). Carbonic acid converts the nutrients of the soil to the soluble form for absorption by roots (Berkeley 1971). Organic manuring does not harm the rhizosphere flora. Moreover, application of rhizospheric bacteria in the soil increases the crop yield (Gunapala and Scrow, 1998).

The contribution of nitrogen fixing bacteria to tobacco growth is not clear. Therefore, the findings present a clear evidence of promoting growth of tobacco plant by non-nitrogen fixing rhizospheric bacteria.

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