Indian Journal of Agricultural Sciences **84** (1): 96–99, February 2014/Article

Genotype dependent variation in native and inoculated soil microorganisms of FCV tobacco rhizosphere in vertisols and alfisols

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Received: 5 January 2013; Revised accepted: 14 June 2013

ABSTRACT

Present study conducted during 2008-09 addresses the acquisition of nutrients from soil by tobacco (*Nicotiana tabacum* L.) plants with specific emphasis on the genotype and soil type with reference to structural and functional characteristics of roots that influence the availability and uptake of P and N. Objective is to explore the impact of tobacco genotypes VT-1158 and Siri in vertisols where as 16/108, and Kanchan in alfisols on the activity of beneficial microorganisms such as *Azotobacter, Azospirillum* and *Pseudomonas* along with native microflora of the rhizosphere. After 45 DAT, the bacterial population was highest in number and it ranged from 7.02 to 10.90 in tobacco rhizosphere of vertisols and alfisols, while fungi were lowest in number which ranged from 3.92×10^3 to 5.30×10^3 cfu/g soil in the rhizosphere soil of vertisols var VT-1158 and Siri respectively. At 90 DAT microbial population declined in vertisols and alfisols of all the varieties. Inoculation of *Azospirillum* and *Pseudomonas* along with application of RDF resulted in maximum viable cell number of 5.89×10^6 cfu/g and 5.90×10^6 cfu/g soil in case of vertisols rhizoshpere of var VT-1158, while 5.95×10^6 cfu/g and 6.47×10^6 cfu/g soil in the rhizosphere of alfisols tobacco respectively after 45 DAT. Inoculation of bacteria either as monoculture or mixed biofertilizer resulted in almost 10-30 times increase in microbial population of the inoculated bacteria; however, their population decreased after 90 DAT. Mixed biofertilizer (*Azotobacter, Azospirillum* and *Pseudomonas*) had a prolonged effect on plant parameters tested and showed a higher nutrient (N and P) content.

Key words : *Azospirillum*, Azotobacter, Pseudomonas, Rhizosphere, Tobacco

Rhizosphere is a complex environment where roots interact with physical, chemical and biological properties of soil. Structural and functional characteristics of roots contribute to rhizosphere processes and both have significant influence on the capacity of roots to acquire nutrients. Depending on genotype of FCV tobacco (*Nicotiana tabacum* .L) and soil type, roots interact extensively with soil microorganisms which show further impact on tobacco plant nutrition and leaf grade outturn either directly by influencing nutrient availability and uptake or indirectly through plant (root) growth promotion (Subhashini, 2013). Particularly, the importance of soil microorganisms and their interactions with roots in relation to nutrient availability is considered along with their associated mechanisms of plant growth promotion. It has become increasingly evident that root interactions with soil microorganisms are intricate and involve highly complex communities that function in very heterogeneous environments (Giri *et al*. 2005). Present study complements previous reports that have specifically focused on either plant-based traits or mechanistic processes associated with P and N uptake (Bucher 2007, Jackson *et al*. 2008). Although the rhizosphere is important for the efficient uptake of a wider range of macro and micronutrients, the study specifically focuses on N and P which are key nutrients that limit sustainable tobacco production (Subhashini and Padmaja, 2010). Furthermore, roots interact with diverse populations of soil microorganisms which have significant implication on growth and nutrition (Brimecombe *et al*. 2007). Microbial interactions with roots may involve either endophytic or free living microorganisms and can be symbiotic, associative or casual in nature. Associative and free-living microorganisms contribute to the nutrition of plants through a variety of mechanisms including direct effects on nutrient availability (e.g. N_2-fixation by diazotrophs and P-mobilization by many microorganisms), enhancement of root growth (i.e. through plant growth promoting rhizobacteria, or PGPR), as antagonists of root pathogens (Subhashini, 2011). Present study deals with the features of FCV tobacco rhizosphere that are important for nutrient acquisition from soil with specific emphasis on the native and inoculated microorganisms that influence the availability and uptake of phosphorus and nitrogen. The interaction of roots with soil microorganisms, in particular with nitrogen

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fixing and phosphorus solubilising plant growth promoting rhizobacteria were studied.

MATERIALS AND METHODS

Strains of *Azotobacter chroococcum, Azospirillum* and *Pseudomonas* were obtained from Department of Microbiology, TNAU, Coimbatore. These were grown on Jensen nitrogen, malate, and Kings B agar media respectively. Rhizosphere soil samples of both the locations Traditional Black Soils (TBS) known as vertisols and Northern Light Soils (NLS) known as alfisols were collected from all the four varieties. The soil in vertisols is clay (with a pH of 7.6, E.C-2.95, O.C - 0.41%, available P - 14 kg/ha, K - 633 kg/ ha) and in alfisols the soil was loamy sand with a pH of 5.6, organic carbon 0.54%, available P 40.62 kg/ ha and available K 392 kg/ha, electrical conductivity 0.10 ds/m, chlorides 24ppm. Viable counts of bacteria, fungi, actinomycetes, *Azotobacter, Azospirillum* and *Pseudomonas* were determined on nutrient agar, potato dextrose agar, Kenknight, Jensen, malate and King's B media respectively. Plates were incubated at 30°C. Tobacco varieties used in the experiment were VT-1158 and Siri in clay soils whereas 16/108 and Kanchan were used in light soils. Recommended doses of fertilizers (RDF) viz., 50 kg N for vertisols and 10 kg each of KP were used per hectare. Seedlings were kept at a depth of 2 cm and inoculated with 1 ml broth of bacterial strain per seedling in all the treatments. The treatments used with both the soils and four genotypes were control, 100% RDF, *100% RDF + Azotobacter,* 100% *RDF + Azospirillum,* 100% RDF + *Pseudomonas* and 100% RDF + mixed biofertilizer. Sampling was done after 45 and 90 DAT. Plants were uprooted and 10 g of rhizospheric soil of each treatment was suspended in 90 ml sterilized distilled water. After appropriate serial dilutions of each sample, plating was carried out with the respective media corresponding to three cultures. Viable cell counts of *Azotobacter* and *Pseudomonas* were taken on Jensen and King's B media respectively, while viable counts of *Azospirillum* were taken on malate media supplemented with antibiotics. Plates were incubated at 30°C till the appearance of colonies on the plate. Plant height, plant dry weight, nutrient contents of leaf nitrogen (AOAC, 1950), phosphorus by vanidomolybdate method were determined 90 DAT.

RESULTS AND DISCUSSION

Native microbial population in rhizosphere of alfisols and vertisols

There was a significant difference in the population of total bacteria, fungi, actinomycetes, *Bacillus, Azotobacter, Azospirillum* and *Pseudomonas* in four varieties tested. However, after 45 DAT, the bacterial population was highest in number and it ranged from 7.02 to 10.90 in tobacco rhizosphere of vertisols and alfisols, while fungi were lowest in number which ranged from 3.92×10^3 to 5.30×10^3 cfu/ g soil in the rhizosphere soil of vertisols var VT-1158 and Siri respectively (Fig 1). At 90 DAT the microbial population declined in vertisols as well as alfisols tobacco rhizosphere soil in all the varieties. This may be due to abundent release of root exudates at initial stages of plant growth which support the multiplication of rhizosphere microorganisms but at later stages of growth, amount of root exudates decreased resulting in decrease in viable counts of microorganisms in the rhizosphere (Narula *et al*. 2009). The significant differences in the number of microorganisms in vertisols and alfisols tobacco rhizosphere at initial stages of growth are because of release of growth promoting substances as root exudates beneficial to microorganisms thus affecting their population in the rhizosphere (Parmar and Dufresne, 2011). However, at later stages of growth, their population was almost similar in the rhizosphere of both soils because of less concentration of these substances in the rhizosphere of clay and light soils (Kamlesh *et al*. 2010).

Table 1 Survival and establishment of bioinoculants under field conditions in the rhizosphere of tobacco genotypes grown in vertisols and alfisols

Microorganisms	Viable counts (× 10^6 CFU/g soil)							
	Vertisols				Alfisols			
	45 days		90 days		45 days		90 days	
	VT-1158	Siri	VT-1158	Siri	16/108	Kanchan	16/108	Kanchan
Control	4.05	4.28	3.91	4.02	3.53	3.76	3.39	3.59
RDF	3.80	4.23	3.78	3.81	3.41	3.58	3.29	3.49
RDF+*Azotobacter*	4.59	5.62	4.23	5.47	4..75	5.76	4.61	5.62
RDF+*Azospirillum*	5.89	5.95	5.71	5.75	5.75	5.91	5.54	5.65
RDF+ *Pseudomonas*	5.90	6.47	6.20	5.73	5.70	5.93	5.41	5.73
RDF + *Azoto* + *Azospirillum* +*Pseudomonas*	5.19	5.70	5.10	5.41	5.02	5.41	4.95	5.29
SEM ±	0.04	0.18	0.04	0.04	0.02	0.02	0.03	0.04
CD (P=0.05)	0.15	0.61	0.13	0.12	0.07	0.07	0.11	0.12
CV (%)	1.48	5.52	1.22	1.25	0.71	0.69	1.23	1.25

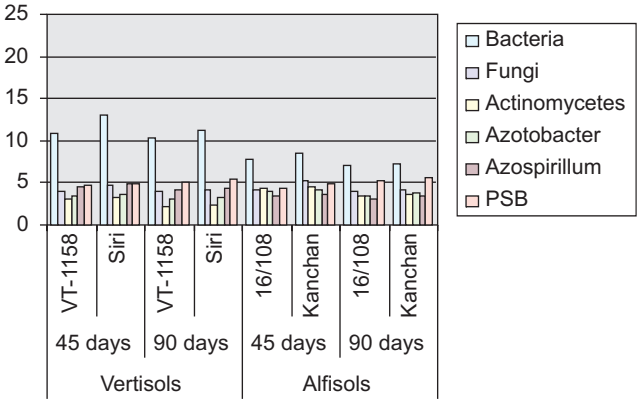

Fig 1 Native microbial population (viable counts as log CFU/g soil) in the vertisols and alfisols grown tobacco under field conditions in the rhizosphere of tobacco genotypes

Establishment of inoculated strains of Azotobacter, Azospirillum and Pseudomonas in the rhizosphere of black soil and light soil

Field experiment was carried out to study the survival and establishment of inoculated microbial population.Strains of *Azotobacter*, *Azospirillum* and *Pseudomonas* were used to inoculate the rhizosphere. Data compiled in Table 1 show that inoculation of *Azotobacter / Azospirillum* / *Pseudomonas* alone as monoculture or as mixed bioinoculants to RDF had more rhizospheric soil population as compared to control alone or RDF alone. However, maximum population of *Azotobacter* was observed after 45 DAT both in rhizosphere of vertisols and alfisols of tobacco var VT-1158 which has 4.59×10^6 cfu/g and 5.62×10^6 cfu/g soil and there was slight decrease in their population after 90 DAT. Moreover, the *Azotobacter* strain established better in the var Siri and showed ten times more population in rhizospheric soil of alfisols as compared to vertisols after 45 DAT. At 90 DAT, the rhizosphere soil of vertisols and alfisols tobacco showed almost similar population of *Azotobacter* strain. Similar results were also obtained with *Azospirillum* and *Pseudomonas* when rhizosphere soil samples were plated on malate and King's B media respectively. Inoculation of *Azospirillum* and *Pseudomonas* along with application of RDF resulted in maximum viable cell number of 5.89×10^6 cfu/g and 5.90×10^6 cfu/g soil in case of vertisols rhizoshpere of var VT-1158, while 5.95×10^6 cfu/g and 6.47×10^6 cfu/g soil in the rhizosphere of alfisols tobacco respectively after 45 DAT (Table 1). These results suggest that inoculation of these bacteria either as monoculture or mixed biofertilizer resulted in almost 10-30 times increase in microbial population of the inoculated bacteria; however, their population decreased after 90 DAT. The significant difference in microbial population in the rhizosphere soil of vertisols and alfisols grown tobacco initially up to 45 DAT was due to the release of root exudates at initial stages of growth that affected the microbial population but at later stages of growth the concentration and

Table 2 Effect of Bioinoculants on plant height, dry weight, N and P uptake in tobacco genotypes of vertisols and alfisols

Treatment	Plant height (cm) after 90 DAT				Plant dry weight (g) after 90 DAT				Percent N uptake (90 DAT)				Percent P uptake (90 DAT)			
	Vertisols		Alfisols		Vertisols		Alfisols		Vertisols		Alfisols		Vertisols		Alfisols	
	VT-1158	Siri	16/108	Kan-chan	VT-1158	Siri	16/108	Kan-chan	VT-1158	Siri	16/108	Kan-chan	VT-1158	Siri	16/108	Kan-chan
Control	92	98	87	92	40	43	39	45	1.92	1.99	1.89	1.91	0.19	0.20	0.19	0.20
RDF	107	117	102	112	91	93	87	93	1.96	2.06	1.93	2.03	0.21	0.22	0.21	0.22
RDF+*Azoto*	112	121	106	116	91	95	88	95	2.13	2.26	2.10	2.20	0.22	0.23	0.21	0.23
RDF + *Azospirillum*	115	122	115	122	93	96	87	97	2.10	2.26	2.20	2.23	0.22	0.23	0.22	0.22
RDF + *Pseudomonas*	120	127	118	125	95	100	92	102	2.10	2.23	2.03	2.10	0.23	0.24	0.23	0.24
RDF + *Azoto* + *Azospirillum* + *Pseudomonas*	131	137	135	142	105	109	98	108	2.33	2.43	2.30	2.35	0.24	0.25	0.24	0.25
SEM ±	1.70	1.63	1.94	1.93	0.54	0.77	1.19	1.07	0.02	0.05	0.03	0.04	0.004	0.003	0.004	0.01
CD (P=0.05)	5.37	5.15	6.14	6.08	1.69	2.43	3.77	3.38	0.07	0.14	0.08	0.14	0.02	0.009	0.01	0.02
CV (%)	2.61	2.35	3.05	2.83	1.08	1.49	2.52	2.06	1.96	3.70	2.36	3.71	3.74	2.33	3.51	4.19

the effect of root exudates decreased (Bais *et al*., 2006). The environmental factors were also responsible for decrease in viable cell number and their establishment in the rhizosphere of plants as reported by Alizadeh and Ordookhani (2011).

Effect of inoculation of Azotobacter, Azospirillum and Pseudomonas

Studies on the effect of inoculation of *Azotobacter, Azospirillum* and *Pseudomonas* strains on plant height, plant biomass, nitrogen and phosphorus content in vertisols and alfisols grown tobacco showed marginal increase in plant height with the addition of recommended dose of fertilizers both in vertisols as well as alfisols tobacco (Table 2). Inoculation of these strains either as monoculture or mixed bioinoculants further shot up this increase up to 20 to 40% increase in plant height at 90 DAT. Similar trend was observed with plant dry weight, N and P content (Table 2) both in vertisols as well as alfisols grown tobacco. Interestingly, only mixed biofertilizer (*Azotobacter, Azospirillum* and *Pseudomonas*) had a prolonged effect on plant parameters tested and showed a higher nutrient (N and P) content which may be due to the additive effect of all the inoculants present in mixed biofertilizer. Similar results were also obtained by Ordookhani and Zare (2011).

From the present study, it can be concluded that genotype of tobacco had a transient effect on rhizosphere soil microbial population which has not persisted upto 90 days of growth due to which not much difference in the population of native as well as inoculated bacteria was observed both in vertisols and alfisols tobacco rhizosphere soil.

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