MICROFLORA ASSOCIATED WITH PHYLLOPLANE, CAULOPLANE AND RHIZOSPHERE OF TWO POPULAR RUBBER CLONES AT TWO LOCATIONS IN INDIA

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Phylloplane, cauloplane and rhizosphere populations of bacteria, fungi, actinomycetes and yeasts associated with the *Hevea brasiliensis* trees of three age groups (2-3, 5-6 and 10-11 years) and belonging to two popular clones (RRII 105 and PB 260) grown in two locations (Cheruvally and Malankara Estates) in the traditional rubber growing tract of India were evaluated. Bacteria dominated in all the three niches while the population of yeast was the lowest. Rhizosphere harboured the highest and cauloplane the lowest number of microorganisms. There was no significant variation in microflora between the two clones. In general, there was an increase in microbial population with the age of the plantation but with respect to the location there was no general trend. The availability of plant exudates appears to influence the association of microflora on the plant surfaces.

Key words: Age, Cauloplane, Clone, *Hevea brasiliensis*, Microflora, Phylloplane, Rhizosphere.

INTRODUCTION

Enumeration and isolation of beneficial microflora associated with crop plants is an essential pre-requisite for developing them as components in sustainable agriculture. There have been very few attempts at enumeration of microorganisms associated with *Hevea brasiliensis*. These were mainly on rhizosphere microorganisms (Joseph *et al.*, 1988; Kothandaraman *et al.*, 1991; Deka *et al.*, 1992; Jayaratne, 1995; Deka *et al.*, 1998) and a few on phyllosphere microflora (George and Kothandaraman, 1999). Some of the organisms isolated have been evaluated for biological control of *Corticium salmonicolor* (Joseph *et al.*, 1991), *Phellinus noxius* (Jacob *et al.*, 1991; Kothandaraman *et al.*, 1991) and *Phytophthora meadii* (Vanitha *et al.*, 1994) causing diseases on *H. brasiliensis*.

The population of microorganism is likely to vary depending on the plant species, age and environment. The present study aims at enumeration of microorganisms associated with two *H. brasiliensis* clones (RRII 105 and PB 260) in three age groups (2-3, 5-6 and 10-11 years) at two locations, *viz.*, Cheruvally (Pathanamthitta District) and Malankara (Idukki District) Estates in Kerala State, India.

MATERIALS AND METHODS

Leaf, bark and rhizosphere soil samples were collected from the two locations from plantations of the two clones in each of the age groups. The enumeration was carried out during February 2000. For phylloplane microbial studies leaflets collected randomly from four trees were pooled.

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The leaf blades were cut into pieces and 100 cm^2 were used for each replication. For cauloplane studies, bark surface scrapings from an area of 25 cm^2 each from four trees were pooled for each replication. The rhizosphere soil sample consisted of soil adjoining rubber roots collected from four spots along with the feeder roots and pooled together for each replication. Three replications were maintained for each treatment combination to form 18 samples for each location. Dilution plate technique was used for the isolation and enumeration of microorganisms.

Labens medium (Laben, 1969) was used for isolation of phylloplane and cauloplane organisms. The basal medium was amended with cyclohexamide (50 mg/L) and 2, 3, 5 triphenyl dihydro tetrazolium chloride (50 mg/L) for isolation of bacteria. For isolation of fungi the medium was amended with tetracycline hydrochloride (500 mg/L) and for yeasts the pH of the medium was adjusted between 4.4 and 4.8 using 0.1 N sulphuric acid.

The media used for isolation and enumeration of rhizosphere microflora included rose bengal agar (amended with 1% streptomycin) for fungi and yeasts, soil extract agar (10% of 1 g/ml soil-water extract) for bacteria and Kenknight's agar for actinomycetes. Appropriate dilutions were plated for each type of microorganism. The plates were incubated at 28±1°C for 3 to 5 days for bacteria, fungi and yeasts and for 15 days for actinomycetes. The number of individual colonies were counted using a colony counter. Unique colonies of each microorganism were picked, purified and maintained on potato dextrose agar (PDA) (for fungi, actinomycetes and yeasts) and on nutrient agar (for bacteria) for further study. The data were subjected to factorial analysis under randomised block design and the influence of the treatment components examined separately.

RESULTS AND DISCUSSION

The mean population of each of the microorganisms associated with *H. brasiliensis* is shown in Table 1. The bacterial population was higher than all other microbial populations, in all the three zones studied. Among the three niches, rhizosphere had the maximum number of microorganisms. Yeast population was generally lower and it was the least in the cauloplane. Kothandaraman *et al.* (1989) reported that bacteria dominated the rhizosphere microorganisms followed by fungi and actinomycetes in the traditional rubber growing area of India but the relative population of bacteria was much lower (55.1 x 10^4/g soil). Deka *et al.* (1992) reported a higher population of bacteria and actinomycetes from the rhizosphere of *H. brasiliensis* from North East India, a non-traditional rubber growing region. The populations of microorganisms are likely to vary according to the moisture level, climatic conditions, locations, variety and age of the plants. In the present study both the locations were high rainfall areas in the traditional rubber growing tract of India.

Table 1. **Population of microorganisms associated with *H. brasiliensis***

Microorganism	Phylloplane (CFU/cm^2)	Cauloplane (CFU/cm^2)	Rhizosphere (CFU/g)
Bacteria	57 x 10^5	73.4 x 10^5	47.5 x 10^7
Fungi	145 x 10^3	31.3 x 10^3	40.5 x 10^3
Actinomycetes	5 x 10^3	03.9 x 10^3	70.0 x 10^3
Yeasts	49	10	11.6 x 10^2

CFU: Colony forming units

The number of microorganisms observed on the phylloplane are presented in Table 2. Significantly higher populations of bacteria and actinomycetes were found in the phylloplane of clone RRII 105 compared to PB 260. The phylloplane populations of fungi and yeasts did not show any variation between these clones. The population of microorganisms on the phylloplane is known to be influenced by the leaf nutrient content (Joseph, 1998). The nutrients on the leaf surface may be released by the leaves or by other microorganisms (Sarkar and Sammadar, 1982). The leaf nutrient content of rubber is known to vary with clone (Potty *et al.*, 1980), leaf age (Abraham *et al.*, 1997), nutrient status of soil (George *et al.*, 2001) and cultural practices like cover crop establishment (Mathew *et al.*, 1980).

Between the two locations, Malankara had a higher population of phylloplane bacteria, actinomycetes and yeasts. Though both the estates fall under the high rainfall tract, Malankara Estate receives higher rainfall than Cheruvally Estate. Moreover, the atmospheric humidity in Malankara Estate remains higher throughout the year due to its proximity to the Malankara dam reservoir.

Compared to immature (2-3 years) plantations, the actinomycete and yeast population were significantly higher in mature plantations (5-6 and 10-11 years). Bacterial populations did not show such a trend. The population of fungi did not vary significantly with clone and age.

The data on cauloplane microorganisms are presented in Table 3. Among the microbes, the yeast population alone showed significant variation between clones, with clone RRII 105 harbouring more. Between the two locations, on the cauloplane the bacterial population was significantly higher in Cheruvally Estate while the population of yeast was higher in Malankara Estate. Bacterial and actinomycete populations showed significant increase with the age of the plantation. No uniform trend was observed with respect to any of the groups of microorgan-

Table 2. **Microorganisms in the phylloplane of *H. brasiliensis***

Microorganism	Clone			Location			Age of plantation (years)			
(CFU/cm^2)	RRII 105	PB 260	CD	Cheruvally	Malankara	CD	2-3	5-6	10-11	CD
Fungi x 10^3	153.50	137.56	NS	138.33	152.77	NS	140.67	133.00	162.92	NS
Bacteria x 10^5	77.78	35.61	15.78	18.11	95.28	15.78	69.17	33.92	67.00	19.32
Actinomycetes x 10^3	6.17	3.89	1.44	4.06	6.00	1.44	3.33	5.50	6.25	1.78
Yeasts x 10^1	53.89	43.89	NS	14.38	82.94	15.78	20.50	35.92	89.50	19.33

CFU: Colony forming units NS: Not significant

Table 3. **Microorganisms in the cauloplane of *H. brasiliensis***

Microorganism	Clone			Location			Age of plantation (years)			
(CFU/cm^2)	RRII 105	PB 260	CD	Cheruvally	Malankara	CD	2-3	5-6	10-11**	CD
Fungi x 10^3	31.67	30.78	NS	29.89	32.56	NS	26.17	34.08	33.42	NS
Bacteria x 10^5	85.83	60.89	NS	88.17	58.56	26.99	47.42	65.92	106.75	33.05
Actinomycetes x 10^3	2.72	2.78	NS	2.56	2.94	NS	1.08	3.08	4.08	1.56
Yeasts x 10^1	12.78	6.72	2.93	6.28	13.22	2.93	8.75	10.50	10.00	NS

CFU: Colony forming units NS: Not significant

isms. Among the plant zones, the cauloplane had the least population of microorganisms perhaps due to the poorer substrate / nutrient availability. There is practically very little exudate on the stem. Lack of moisture may be another limiting factor. The beneficial effect of cauloplane saprophytes on prevention of stem diseases has been reported (Bier, 1963). Detailed studies on cauloplane microorganisms of trees are rare and limited to bark fungi (Garner, 1967).

The populations of the different microorganisms in the rhizosphere are presented in Table 4. There was no significant difference in the rhizosphere microorganisms between the two clones studied except for bacteria. The bacterial population was significantly high in clone RRII 105 compared to PB 260. Between the two locations, significantly higher actinomycete population was observed in Malankara Estate than in Cheruvally Estate while the population of rhizosphere yeasts was more in Cheruvally Estate. The populations of fungi and bacteria did not show any significant variation between the two locations. When immature plantations (2-3 years old) were compared with the mature (10-11 years old) ones, significantly higher populations of fungi and bacteria were observed in the rhizosphere of the latter. While the fungal population remained low during the immaturity period (up to 6 years) the bacterial population increased with the canopy coverage (after 3 years). Age of the plantation did not result in significant variation in the population of actinomycetes and yeasts in the rhizosphere.

It is well known that the microbial population in the rhizosphere is influenced by the roots (Clark, 1949). The microbial population in the rhizosphere is high due to the presence of root exudates consisting of aminoacids, sugars, organic acids, mucilage, sloughed off root cells and other substances (Griffin *et al.*, 1976; Rovira *et al.*, 1979). Earlier studies on rhizosphere microflora of *Hevea* have reported high population of bacteria and low population of yeasts (Joseph *et al.*, 1988; Deka *et al.*, 1992). The results of the present study uphold the observations made earlier. The present study also confirms their finding that the rhizosphere population of actinomycetes was higher than that of fungi.

Though the population of each group of microorganisms was found to vary with clone, location and age of the plantation, some general trends could be observed from the study. Bacteria were the most dominant microorganism while yeasts the least dominant in all the three niches. Rhizosphere harboured the highest and cauloplane the lowest microbial population. While the clones and locations did not generally influence the microflora, increase in age of plantation favoured their association. The nu-

Table 4. **Microorganisms in the rhizosphere of *H. brasiliensis***

Microorganism	Clone			Location			Age of plantation (years)			
(CFU/g)	RRII 105	PB 260	CD	Cheruvally	Malankara	CD	2-3	5-6	10-11**	CD
Fungi x 10^3	39.67	41.00	NS	41.56	39.11	NS	35.08	32.17	53.75	13.56
Bacteria x 10^7	53.39	41.56	10.96	52.22	42.72	NS	36.25	52.33	53.83	13.42
Actinomycetes x 10^3	61.70	80.00	NS	51.70	90.00	22.89	62.50	69.20	80.80	NS
Yeasts x 10^2	12.06	11.11	NS	14.83	8.33	3.17	10.92	10.67	13.17	NS

CFU: Colony forming units NS: Not significant

trient availability through plant exudates appears to influence microbial colonization.

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