(H_3–3, 5) 5.30 (H–5), 5.18 (H–1), 5.28 (H–7, 1.1 Hz).

The UV and IR spectral data of the factor EM_1 and EM_2 show common skeleton and functionalities, except for mass values, where the difference of one hydroxyl group is indicated clearly in factor EM_2. When we compare the NMR data and count the positions of various hydroxyls including that of the one at 4–position in ingenol moiety, (free in acetylated ingenol)[6], the signals of 4–OH group are present in ingenol 3, 5, 20–triacetate (factor EM_1) but not in EM_2 at 3.28 position. It appears that EM_2 is devoid of a hydroxyl function and hence is called 4–deoxy ingenol 3, 5, 20 triacetate and the parent diterpene will be similarly known as 4–deoxy ingenol[7].

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A PRELIMINARY STUDY ON ROOT ASSOCIATED DIAZOTROPHS OF COTTON

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THE rhizosphere studies conducted with field grown cotton showed a greater number of total (11×10^7 cells/g) and diazotrophic (2×10^5 cells/g) bacteria associated with roots of indigenous cotton. The counts of such bacteria were highest in the rhizoplane and lowest in root macerate samples. Cotton var. H777 and G27 had *Klebsiella* sp. and *Enterobacter* sp. as their root associated diazotrophs. However, more work is required to understand the contribution of these organisms for the improvement of cotton.

Among the rhizosphere organisms, diazotrophic bacteria are of major interest because of their active involvement in crops productivity[1,2]. However, the root associated diazotrophs of cotton have not been studied so far. Therefore, an attempt was made to find out the number and types of these bacteria with cotton roots.

Cotton varieties H777 (*Gossypium hirsutum*) and G27 (*Gosaypium arboreum*) were grown at the university farm following recommended agronomical practices. The plants were uprooted at the preflowering stage. Rhizosphere samples along with roots were collected from 3 different locations for each variety. These were used for total (dilution plating) and most probable number (MPN) of bacteria (acetylene reduction method) from rhizosphere (RS), rhizoplane (RP) and root macerate (RM). Nitrogen-deficient malate medium[3] was used for counts and nitrogenase activity. The MPN was estimated by comparing the results with standard tables[4].

The malate medium plates used for total counts and streaked from MPN positive tubes were taken for isolating the root associated diazotrophs based on colony morphology. A total of 21 isolates (11 from H777 and 10 from G27) were checked for acetylene reduction activity (ARA) on solid as well as semisolid media. The two isolates, 8C (var. H777) and DC8 (var. G27) showing highest ARA were selected for subsequent studies. These were identified by standard procedures[5].

The samples analysed for total and diazotrophic bacteria associated with roots showed greater number with G27 than H777 (table 1). The counts were greater in the rhizoplane compared to rhizosphere, may be due to the availability of more photosynthates near the root surface. The surface-sterilized roots showed very few bacteria. These results show that indigenous cotton has greater potential to harbour nitrogen-fixing and other bacteria to meet the nutritional requirements. The other possible explanation may be the long association of rhizosphere microflora and indigenous cotton which helped in developing a mechanism to live together.

Table 1 *Number of root associated bacteria of cotton*

Cotton variety	Sample	Root bacteria					
		Rhizosphere		Rhizoplane		Root macerate	
		Total	MPN	Total	MPN	Total	MPN
H777	I	27	2	93	4	–	–
	II	42	–	69	4	–	–
	III	35	–	72	2	–	–
G27	I	45	4	108	9	2	–
	II	63	2	92	5	0	–
	III	88	–	89	15	1	–

Total × 10^6 cells/g; MPN × 10^4 cells/g.

Table 2 *Isolation of root associated diazotrophs of cotton*

Cotton variety	Sample		Isolate	Acetylene reduction activity (n moles C_2H_4/ 24 hr/ tube)	
				Solid medium	Semisolid medium
H777	Rhizosphere	I	1C	3.2	ND
			2C	2.4	ND
		II	3C	ND	ND
		III	4C	ND	22.2
			5C	6.9	ND
	Rhizoplane	I	6C	16.6	25.9
		II	7C	ND	ND
			8C	30.0	24.0
			9C	ND	103.4
		III	10C	ND	ND
			11C	9.5	ND
G27	Rhizosphere	I	1DC	ND	6.3
			2DC	181.9	18.0
		II	3DC	ND	ND
		III	4DC	ND	ND
			5DC	3.8	15.3
	Rhizoplane	I	6DC	90.0	ND
			7DC	ND	ND
		II	8DC	156.4	34.7
			9DC	4.3	ND
		III	10DC	ND	ND

ND–Not detectable.

Bacteria picked up from total and MPN counts were analysed for ARA on solid and semisolid medium (table 2). The rhizoplane isolates showed greater nitrogen fixation than rhizosphere in both types of cotton. Root macerate isolates did not show ARA. The bacteria showing ARA had variations in O_2 requirements. These results indicate the diversity of rhizosphere bacteria. Results of similar type have also been obtained with barley, wheat, pearl millet and sorghum[6,7].

The isolates 8C and 8DC showing highest ARA were selected for subsequent studies. Isolate 8C was short rods, singly or in pairs, gram-negative, non-motile and aerobic bacteria. It showed positive methyl red, Voges Proskauer, catalase, nitrate reductase tests and utilization of citrate, ammonia, glucose, arabinose, maltose, mannitol, sucrose, xylose and gluconate. The test was negative for H_2S production, gelatin, indole and arginine utilization. The isolate 8DC was motile rods, gram-negative and capsules forming bacteria. This showed positive Voges Proskauer, gelatin hydrolysis tests and utilization of catalase, nitrate reductase and utilization of citrate, acetate, lactose, maltose, sucrose, xylose and gluconate. The oxidase, methyl red, H_2S production and indole tests were negative. Comparing these characteristics with Bergery's Manual, 8C and 8DC were identified as *Klebsiella* sp. and *Enterobacter* sp., respectively.

The present results show that indigenous cotton has more of total and N_2-fixing bacteria with the roots, which may be of various types. The types of bacteria associated with roots of a particular plant species are determined mainly by the plants; however environmental factors also play an important role. The *Klebsiella* sp. were found associated with exotic cotton, whereas, *Enterobacter* sp. with indigenous type. This being a preliminary study, more work is needed to understand the microbiology of cotton rhizosphere and the factors involved in such an association. The possibility of using root associated nitrogen-fixing bacteria for crop productivity is yet to be studied.

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IMPROVEMENT OF CARRIER MATERIAL FOR DEVELOPMENT OF RHIZOBIAL INOCULANTS

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ONE of the fundamental criteria necessary for longer survival of rhizobia in carrier is its high moisture-retaining capacity. There is considerable evidence to show how moisture loss from carrier adversely affects the survival of rhizobia[1,2]. Moisture loss may also tend to increase concentration of harmful soluble salts in carrier[3]. Recently, a change in packing material of inoculants from cans and bottles with lids/stoppers/or screw caps to pliable low

Table 1 *Survival of red gram Rhizobium (cowpea sp.) ARS-83 in charcoal-soil (3:1) carrier amended with soymeal or gelatin at 28–30°C (Av. of 3 replications)*

	No. of viable cells $\times 10^8$/g of carrier			
	Weeks after inoculation			
Carrier	0	12	24	Mean
Carrier	90.00 (29.00)	3.75 (14.00)	0.02 (4.00)	31.26 (15.67)
Carrier + soymeal (1%)	95.00 (28.33)	9.00 (16.00)	0.08 (8.00)	34.69 (17.44)
Carrier + soymeal (2%)	95.00 (28.72)	13.00 (17.60)	0.10 (10.40)	36.03 (18.91)
Carrier + soymeal (5%)	100.00 (27.81)	16.00 (20.67)	0.86 (17.40)	38.95 (21.96)
Carrier + soymeal (10%)	90.00 (30.80)	46.50 (29.00)	3.10 (24.00)	46.53 (27.93)
Carrier + gelatin (1%)	94.67 (29.84)	1.40 (14.00)	0.06 (7.60)	32.04 (17.15)
Carrier + gelatin (2%)	95.00 (27.22)	2.00 (20.00)	1.93 (16.00)	32.97 (21.07)
Mean	94.24 (28.82)	13.09 (18.75)	0.88 (12.49)	

	S. Em	*C. D. at 5%*
Treatment (*T*)	1.05 (0.65)	3.02 (1.86)
Period (*P*)	0.69 (0.42)	1.97 (1.22)
$T \times P$	1.83 (1.13)	5.23 (3.22)

Figures in parentheses are moisture contents (%) in carrier.

ND–Not detectable.