

**EFFECT OF SEED AND SEEDLING EXUDATE COMPOUNDS
OF LEGUMES AND NON-LEGUMES ON GROWTH
AND CHEMOTAXIS OF RHIZOBIA**

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**DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE
1995**

**EFFECT OF SEED AND SEEDLING EXUDATE COMPOUNDS
OF LEGUMES AND NON-LEGUMES ON GROWTH
AND CHEMOTAXIS OF RHIZOBIA**

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Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfilment of the requirements
for the award of the Degree of
Master of Science
in
AGRICULTURAL MICROBIOLOGY

BANGALORE

MARCH 1995

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Affectionately Dedicated to
My Beloved Parents

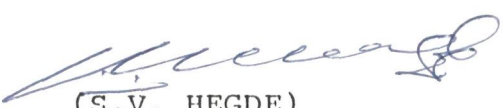
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UNIVERSITY OF AGRICULTURAL SCIENCES, BANGALORE

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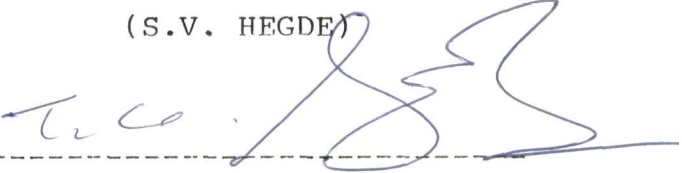
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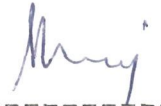
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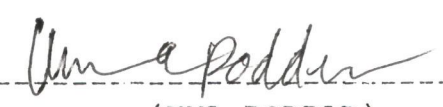
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ACKNOWLEDGEMENT

I express my gratitude to Dr.S.V. Hegde, Professor of Agricultural Microbiology and Chairman of my Advisory Committee for his valuable guidance throughout the course of this study and for his critical perusal of this manuscript.

My thanks are also due to Dr.T.K.Siddarama Gowda, Professor of Agril Microbiology, Dr.J. Raj, Associate Professor of Agril Microbiology and Dr.Uma Poddar, Associate Professor of Biochemistry for having served as members of my Advisory Committee and for their counsel and help during the course of this investigation.

To my friends, Indira, Pragathi, Padmavathi, Tilak, Jones and Lakshminarayan whose friendships have greatly influenced my work, I am most grateful. I also extend my thanks to Kumaran and Wajeed for their co-operation and help during my research work. Lakshminarayan also deserves a special mention for having taken up the computer processing of the thesis and giving momentum for its conclusion.

I owe all my success to my parents and brother and I am deeply indebted to them for their moral and financial support during my study.

Place: Bangalore
Date : March 6th, 1995.


[LATHA P.C.]

CONTENTS

CHAPTER	TITLE	PAGE NUMBER
I	INTRODUCTION	1 - 3
II	REVIEW OF LITERATURE	4 - 40
III	MATERIAL AND METHODS	41 - 47
IV	EXPERIMENTAL RESULTS	48 - 70
V	DISCUSSION	71 - 82
VI	SUMMARY	83 - 84
VII	REFERENCES	85 - 103

LIST OF TABLES

TABLE	TITLE	PAGE NUMBER
1	Carbohydrates detected in the root exudates of various plant species	9a - 9d
2	Amino acids detected in the root exudates of various plant species	10
3	Organic acids found in the root exudates of various plant species	11
4	Growth (log number of colony forming units of rhizobia/ml) of rhizobial strains in the root exudate of legume seedlings	49
5	Growth (log number of colony forming units of rhizobia/ml) of rhizobial strains in the root exudate of non-legume seedlings	50
6	Chemotactic response (relative response) of <u>Rhizobium</u> strains towards seed leacheates of legumes	55
7	Chemotactic response (relative response) of <u>Rhizobium</u> strains towards seed leacheates of non-legumes	55
8	Inhibition of rhizobia by diffusates from germinating legume and non-legume seeds	59
9	Chemotactic response (relative response) of <u>Rhizobium</u> strains towards root exudates of legumes	63
10	Chemotactic response (relative response) of <u>Rhizobium</u> strains towards root exudates of non-legumes	63
11	Amino acids present in the seed leacheates of legumes and non-legumes	67
12	Amino acids present in the root exudates of legumes and non-legumes	69

LIST OF FIGURES

FIGURE	TITLE	B/W PAGES
1	Growth (log number of colony forming units of rhizobia/ml) of rhizobial strains in the root exudate of legume seedlings	49 - 50
2	Growth (log number of colony forming units of rhizobia/ml) of rhizobial strains in the root exudate of non-legume seedlings	50 - 51
3	Chemotactic response (relative response) of <u>Rhizobium</u> strains towards seed leacheates of legumes	55 - 56
4	Chemotactic response (relative response) of <u>Rhizobium</u> strains towards seed leacheates of non-legumes	55 - 56
5	Inhibition of rhizobia by diffusates from germinating legume and non-legume seeds	59 - 60
6	Chemotactic response (relative response) of <u>Rhizobium</u> strains towards root exudates of legumes	63 - 64
7	Chemotactic response (relative response) of <u>Rhizobium</u> strains towards root exudates of non-legumes	63 - 64

LIST OF PLATES

PLATE	TITLE	B/W PAGES
1	Collection of root exudates	42 - 43
2	Inhibition of rhizobial strains by chickpea	59 - 60
3	Inhibition of rhizobial strains by cowpea	59 - 60
4	Inhibition of rhizobial strains by soybean	59 - 60
5	Inhibition of rhizobial strains by groundnut	59 - 60
6	Inhibition of rhizobial strains by maize	59 - 60
7	Inhibition of rhizobial strains by ragi	59 - 60
8	Inhibition of rhizobial strains by sunflower	59 - 60
9	Inhibition of rhizobial strains by cotton	59 - 60

INTRODUCTION

I. INTRODUCTION

The legume-Rhizobium symbiotic association is estimated to fix 80 million tonnes of nitrogen annually from the vast free supply of nitrogen in the air, thus having a great relevance to sustainable agriculture, economy of nations and ecological considerations of the environment. This association is one of the finest examples of plant microbe beneficial interactions in nature evolved from time immemorial. The legume root nodule is a new organ produced as a result of the interactions which acts as the nature's factory to manufacture ammonia from molecular nitrogen.

The legume-Rhizobium interaction resulting in the formation of root nodule involves a series of steps proceeding in an orderly manner which are controlled by several genes contained in the host as well as the microsymbiont Rhizobium. Recent studies have thrown much light on the mechanism of interactions in different steps of nodulation, identifying the gene and gene products involved in the phenotypic expression.

From the time the seed is sown and germination occurs there is known to begin a series of signal exchanges between the host and bacterium which are known to be involved in chemotaxis (attraction), colonization, adhesion, root hair

curling, specificity of recognition, nodule initiation, nodule development, etc. The whole process may be completed in 10-15 days after germination of the seeds under optimum conditions. Much of the signal compounds of the host are thus believed to be present in the exudates of germinating seed and seedlings. The composition of legume root exudates thus have profound influence on the success of nodulation and symbiosis.

Legume root exudates contain a variety of substances which undoubtedly serve as carbon and nitrogen sources for the root nodule and other bacteria. The root exudates may also provide specific growth factors required by auxotrophic rhizobia, e.g. homoserine in pea root exudate is known to specifically attract Rhizobium leguminosarum. The members of the genus Rhizobium exhibit a marked specificity for the host which they invade and nodulate and the chemical and physiological basis of such specificity is now becoming clearer. The tropical legumes show comparatively less specificity to Bradyrhizobium and it could be interesting to compare the root exudate compounds of tropical and temperate legumes.

In Karnataka state and elsewhere in the country pulses (grain legume crops) have always occupied an important place in different cropping systems along with cereals and non-legume crops. The root exudate compounds of both

cereals/non-legumes as well as legumes might play an important role in the early stages of nodulation and symbiotic nitrogen fixation. Not much information is available on the effect of the root exudates of important legume and non-legume crops on establishment of early symbiosis between legumes and rhizobia. Therefore, the present study was undertaken with the following objectives:

1. To examine the effect of legume seed leacheate, germinating seed exudate and seedling root exudate on the growth and chemotaxis of homologous and non-homologous rhizobia.
2. To study the effect of non-legume crop seed leacheate, germinating seed exudate and seedling root exudate on the growth and chemotaxis of rhizobia.
3. To characterize the amino acids of the seed leacheates and root exudates.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

2.1 RHIZOBIUM SYMBIOSIS

Rhizobium, Bradyrhizobium and Azorhizobium, collectively referred to as rhizobia are bacterial symbionts, which infect the root of legumes inducing the formation of symbiotic root nodule which fixes atmospheric nitrogen. The ability of plants to establish nitrogen fixing symbiosis with rhizobia is restricted to legumes, with one exception, the genus Parasponia of the Ulmaceae family (Young and Johnston, 1989). The symbiosis is also very specific within legumes, the host range being precisely determined for any particular rhizobial strain. However, the degree of specificity varies greatly among the rhizobia. Rhizobium meliloti strains elicit nitrogen fixing nodules only on the species of the genera Medicago, Melilotus and Trigonella, R. trifolii only on the species of Trifolium, while most isolates of R. leguminosarum even differentiate between genotypes, nodulating European pea varieties, but not certain peas from Afghanistan (Denaire et al., 1992). In contrast, some tropical Bradyrhizobium strains nodulate legumes in different tribes and subfamilies (Young and Johnston, 1989) while Rhizobium sp. strain NGR 234 nodulates at least 35 legume genera (Denaire et al., 1992).

The formation of nitrogen fixing nodules by rhizobia upon legumes is a complex and highly organized process. The process of nodulation requires a series of interactions between plant and Rhizobium where each interaction is a necessary step in the highly coordinated pathway (Hedges and Messens, 1990). The normal pathway for nodulation has been outlined as arrival in the root zone, recognition at the root, attachment to the root hairs, root hair curling, infective thread formation within root hairs, growth of the thread towards the inner root cortex, initiation of nodule meristem and nodule organogenesis (Schmidt, 1979; Debelle et al., 1986; Sprent, 1989). These steps are thought to proceed with increasing levels of specificity and decreasing levels of reversibility. An exchange of signal molecules between the host legumes and the rhizobia is necessary for the initiation, development and maintenance of symbiosis. It is thought that the plant and the Rhizobium partner recognize and affect the each other by secreting specific substances (Broughton, 1978; Solheim and Paxton, 1981). The legume rhizosphere, where the exchange of signals between the legumes and the rhizobia occurs for the recognition and preinfection events, has been intensively studied.

2.2 THE RHIZOSPHERE

The rhizosphere is that narrow zone of soil subject to the influence of living roots (Curl and Truelove, 1986).

The total rhizosphere environment is determined by an interaction of soil, the plant and the organisms associated with the root (Lynch, 1987). Events that occur in the developing legume rhizosphere are critical for the establishment of the legume Rhizobium-symbiosis. Exudation in the rhizosphere by legumes is thought to exert effects that are central for the initiation of the symbiosis as the colonization of the roots of seedlings by Rhizobium depends to a great extent on the seed and the root exudates, the major sources of nutrition and energy in the rhizosphere.

2.3 ROOT EXUDATES

Root exudates are substances which are released in to the surrounding medium by healthy intact plant roots (Rovira, 1969). Root exudates contain a variety of chemically different compounds like sugars, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavones, enzymes and miscellaneous compounds such as auxins, microbial growth stimulators, inhibitors, etc. (Rovira, 1965; Malajczuk, 1983; Curl and Truelove, 1986). This loss of organic compounds from the plant roots is a passive process due to the leakage of materials through cell walls, accidental aberrations and it is also influenced in part by plant genotype and physiology (Hedges and Messens, 1990).

Rhizosphere effect is also due to other losses arising from the plant root-like the secretion of polymeric carbohydrates and enzymes by the roots, release of lysates due to autolysis of older cells and evolution of gases such as ethylene and CO_2 (Curl and Truelove, 1986; Lynch, 1990).

2.4 COMPOSITION OF THE ROOT EXUDATES

In the frame work of the legume-Rhizobium study, interest in the exact nature of the root exuded compounds was aroused as they were implicated in the rhizospheric life of Rhizobium. Diverse organic compounds have been found in the root exudates of a variety of crop plants. Most of the compounds are water soluble (Curl and Truelove, 1986), though Rovira and Davey (1974) have classified carbon lost through roots as (a) diffusible (ethylene, carbon dioxide) (b) diffusible volatile water soluble (sugars, amino acids) and (c) non-diffusible exudates. Early recognition that root exudes a variety of sugars and amino acids providing readily available carbon and nitrogen sources for microbial growth led to increased research attention being directed towards these compounds. At least 12 sugars and 25 amino acids have been identified in the exudates of plants.

2.4.1 Sugars

Glucose and fructose are generally the most frequently reported sugars in the root exudates (Vancura, 1964). The various carbohydrates identified in the plant root exudates have been summarized in Table 1.

2.4.2 Amino acids

Since Virtanen et al. (1936) reported that lysine and aspartic acid were excreted by root nodules of legumes, amino acids have become the most extensively studied group of compounds in the root exudates of plants. Amino acids exuded by the root vary greatly within plants. Table 2 lists out the various amino acids detected in the root exudates of different plant species.

2.4.3 Organic acids

Exudate components other than sugars and amino acids have received much less attention though organic acids play a prominent role by affecting rhizosphere pH, cell metabolism and microbial activity (Rovira, 1965). Organic acids are found in relatively large amounts in many plants but have been infrequently reported in root exudates (Table 3).

Table 1: Carbohydrates detected in the root exudates of various plant species

Sugar	Plant	References
Glucose	Alfalfa	Scheffer <i>et al.</i> , 1962 Hamlen <i>et al.</i> , 1972
	Bean	Schroth and Snyder, 1961 Vancura and Hanzlikova, 1972
	Clover	Scheffer <i>et al.</i> , 1962 Smith and Paterson, 1966
	Chickpea	Gitte <i>et al.</i> , 1978 Kush and Dadarwal, 1981 Rai and Prasad, 1986
	Lentil	Claudius and Mehrotra, 1973 Rai, 1987
	Peas	Lundegardh and Stenlid, 1944 Rovira, 1956 Gaworzewska and Carlile, 1982
	Soybean	Barbour <i>et al.</i> , 1991
	Barley	Vancura and Hanzlikova, 1972
	Maize	Vancura, 1967
	Mustard	Bhuvaneswari and Rao, 1957
	Oats	Rovira, 1956
	Rice	Macrae and Castro, 1967
	Ragi	Rangaswami and Balasubaramanium, 1967
	Sugarmaple	Smith, 1969
	Wheat	Lundegardh and Stenlid, 1944 Katznelson <i>et al.</i> , 1955 Vancura and Hanzlikova, 1972 Heinrich and Hess, 1985
Fructose	Alfalfa	Hamlen <i>et al.</i> , 1972 Schroth and Snyder, 1961
	Bean	Vancura and Hanzlikova, 1972
	Clover	Scheffer <i>et al.</i> , 1962 Smith and Paterson, 1966
	Chickpea	Gitte <i>et al.</i> , 1978 Kush and Dadarwal, 1981 Rai and Prasad, 1986
	Lentil	Claudius and Mehrotra, 1973 Rai, 1987
	Peas	Rovira, 1956 Gaworzewska and Carlile, 1982
	Banana	Buxton, 1962
	Maize	Vancura, 1967

Continued

Table 1: Continued

Sugar	Plant	References
Sucrose	Mustard	Bhuvaneswari and Rao, 1957
	Oats	Rovira, 1956
	Sugarmaple	Smith, 1969
	Sugarcane	Mujumdar, 1968
	Sorghum	Bhuvaneswari and Rao, 1957
	Wheat	Vancura and Hanzlikova, 1972
		Heinrich and Hess, 1985
	Bean	Schroth and Snyder, 1961
	Chickpea	Gitte <i>et al.</i> , 1978
		Kush and Dadarwal, 1981
		Rai and Prasad, 1986
	Lentil	Claudius and Mehrotra, 1973
		Rai, 1987
	Peas	Gaworzewska and Carlile, 1982
	Soybean	Barbour <i>et al.</i> , 1991
Xylose	Banana	Buxton, 1962
	Rice	Macrae and Castro, 1967
	Ragi	Rangaswami and Balasubramaniam, 1967
	Sugarmaple	Smith, 1969
	Sugarcane	Mujumdar, 1968
	Bean	Vancura and Hanzlikova, 1972
	Clover	Scheffer <i>et al.</i> , 1962
		Smith and Paterson, 1966
	Chickpea	Gitte <i>et al.</i> , 1978
		Kush and Dadarwal, 1981
		Rai and Prasad, 1986
	Lentil	Claudius and Mehrotra, 1973
		Rai, 1987
	Peanut	Rittenhouse and Hale, 1971
	Barley	Vancura and Hanzlikova, 1972
Galactose	Banana	Buxton, 1962
	Maize	Vancura, 1967
	Mustard	Bhuvaneswari and Rao, 1957
	Pine	Slankis, 1958
	Sorghum	Bhuvaneswari and Rao, 1957
	Wheat	Vancura and Hanzlikova, 1972
		Heinrich and Hess, 1985
	Bean	Schroth and Snyder, 1961
		Vancura and Hanzlikova, 1972
	Clover	Scheffer, 1962
		Smith and Paterson, 1966

Continued

Table 1: Continued

Sugar	Plant	References
	Chickpea	Gitte <u>et al.</u> , 1978 Kush and Dadarwal, 1981 Rai and Prasad, 1986
	Lentil	Claudius and Mehrotra, 1973 Rai, 1987
	Peanut	Rittenhouse and Hale, 1971
	Soybean	Barbour <u>et al.</u> , 1991
	Barley	Vancura, 1967
	Banana	Buxton, 1962
	Maize	Vancura, 1967
	Wheat	Vancura and Hanzlikova, 1972 Heinrich and Hess, 1985
Mannose	Chickpea	Gitte <u>et al.</u> , 1978 Kush and Dadarwal, 1981 Rai and Prasad, 1986
Rhamnose	Peanut	Rittenhouse and Hale, 1971
	Banana	Buxton, 1962
	Bean	Schroth and Snyder, 1961 Vancura and Hanzlikova, 1972
	Lentil	Claudius and Mehrotra, 1973 Rai, 1987
Arabinose	Barley	Vancura and Hanzlikova, 1972
	Sugarmaple	Smith, 1969
	Wheat	Heinrich and Hess, 1985
	Chickpea	Gitte <u>et al.</u> , 1978 Kush and Dadarwal, 1981 Rai and Prasad, 1986
Maltose	Soybean	Barbour <u>et al.</u> , 1991
	Barley	Vancura and Hanzlikova, 1972
	Pine	Slankis, 1958
	Rice	Macrae and Castro, 1967
	Sugarcane	Mujumdar, 1968
	Bean	Schroth and Snyder, 1961
	Lentil	Claudius and Mehrotra, 1973 Rai, 1987
Ribose	Barley	Vancura and Hanzlikova, 1972
	Banana	Buxton, 1962
	Maize	Vancura, 1967
	Bean	Schroth and Snyder, 1961 Vancura and Hanzlikova, 1972
	Clover	Sheffer <u>et al.</u> , 1962 Smith and Paterson, 1966

Continued

Table 1: Continued

Sugar	Plant	References
	Chickpea	Gitte <u>et al.</u> , 1978 Kush and Dadarwal, 1981 Rai and Prasad, 1986
	Lentil	Claudius and Mehrotra, 1973 Rai, 1987
	Peas	Gaworzewska and Carlile, 1982
	Peanut	Rittenhouse and Hale, 1971
	Banana	Buxton, 1962
	Maize	Vancura, 1967
	Sugarmaple	Smith, 1969
	Wheat	Heinrich and Hess, 1985
Raffinose	Bean	Vancura and Hanzlikova, 1972
	Banana	Buxton, 1962
	Sugarcane	Mujumdar, 1968
Saccharose	Bean	Schroth and Snyder, 1961 Vancura and Hanzlikova, 1972
	Maize	Vancura, 1967
Deoxyribose	Bean	Schroth and Snyder, 1961
	Barley	Vancura and Hanzlikova, 1972
	Maize	Vancura, 1967
Cellobiose	Bean	Schroth and Snyder, 1961 Vancura and Hanzlikova, 1972
	Wheat	Vancura and Hanzlikova, 1972 Heinrich and Hess, 1985
Mannitol	Lentil	Claudius and Mehrotra, 1973 Rai, 1987
	Soybean	Barbour <u>et al.</u> , 1991
Dihydroxy acetone	Peanut	Rittenhouse and Hale, 1971
Innositol	Alfalfa	Scheffer <u>et al.</u> , 1962 Hamlen <u>et al.</u> , 1972
Lactose	Lactose	Claudius and Mehrotra, 1973 Rai, 1987

Table 2: Amino acids detected in the root exudates of various plant species

	Amino acids																						
Crop	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	H.Ser	Orn	Leu	Ile	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Trp	Val	References
Alfalfa	+	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	1
Bean	+	-	+	+	-	+	+	+	-	-	+	+	+	-	-	+	-	+	+	+	-	+	2
Chickpea	+	+	-	+	+	+	-	+	+	-	-	+	+	+	-	+	-	+	+	-	+	+	3,4,5
Lentil	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	+	6,7
Pea	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8,9,10,11,12
S.Clover	+	-	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	+	+	-	-	+	13
Soybean	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	+	14
W.Clover	+	-	+	+	-	+	+	-	-	-	-	+	-	-	-	-	-	+	+	+	-	-	15
Cotton	-	+	-	+	+	-	-	+	-	-	-	+	+	+	-	+	+	+	+	+	+	-	16,17
Cucumber	+	+	+	+	+	+	-	+	-	-	+	+	-	-	-	-	-	+	+	+	-	+	18
Banana	+	+	-	+	+	+	+	+	-	-	-	+	+	-	-	-	+	+	+	-	-	-	19
Barley	+	+	+	+	+	-	-	+	-	-	+	+	+	-	-	+	+	+	+	+	-	+	3
Maize	+	-	-	+	-	-	-	+	-	-	-	+	+	+	-	+	+	-	+	+	-	+	18
Oats	+	-	+	+	-	-	+	+	-	-	-	+	-	+	-	-	-	+	-	-	-	-	8
P.grass	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	20
S.beet	+	+	+	+	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-	21
S.cane	+	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	22
S.maple	+	-	-	-	-	+	+	+	-	+	-	+	+	-	+	+	-	+	+	+	-	+	23
S.flower	+	-	+	+	-	+	+	-	-	-	-	+	-	-	-	+	-	+	+	-	-	+	24
Rice	-	-	+	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	25
Wheat	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+	+	-	+	+	+	-	-	26

+ = Present; - = Absent

- | | | |
|---------------------------------|-------------------------------------|-------------------------------|
| 1. Ritcher <i>et al.</i> , 1968 | 10. Sherrod & Domsch, 1970 | 19. Buxton, 1962 |
| 2. Vancura & Hanzlikova, 1972 | 11. Katznelson <i>et al.</i> , 1955 | 20. Kemble & Macpherson, 1954 |
| 3. Gitte <i>et al.</i> , 1978 | 12. Buxton, 1957 | 21. Rai & Strobel, 1968 |
| 4. Kush & Dadarwal, 1981 | 13. Rovira, 1959 | 22. Mujumdar, 1968 |
| 5. Rai and Prasad, 1986 | 14. Barbour <i>et al.</i> , 1991 | 23. Smith, 1969 |
| 6. Claudius & Mehrotra, 1973 | 15. Rovira & Harris, 1961 | 24. Frenzel, 1960 |
| 7. Rai, 1987 | 16. Booth, 1969 | 25. Macrae & Castro, 1967 |
| 8. Rovira, 1956 | 17. Sulochana, 1962 | 26. Heinrich & Hess, 1965 |
| 9. Boulter <i>et al.</i> , 1966 | 18. Vancura, 1967 | |

Table 3: Organic acids found in the root exudates of various plant species

Organic acid	Crop	References
Acetic acid	Wheat Sorghum	Heinrich and Hess, 1985 Bhuvaneswari and Rao, 1957
Propionic acid	Wheat	Heinrich and Hess, 1985
Butyric acid	Wheat	Heinrich and Hess, 1985
Valeric acid	Wheat	Heinrich and Hess, 1985
Malic acid and Malonic acid	Wheat Mustard Sugarmaple Cotton Cicer Lentil Soybean	Heinrich and Hess, 1985 Bhuvanewari and Rao, 1957 Smith, 1969 Kovacs, 1971 Gitte <u>et al.</u> , 1978 Rai, 1987 Barbour <u>et al.</u> , 1991
Citric acid	Mustard Sugarmaple Cicer Soybean	Bhuvaneswari and Rao, 1957 Smith, 1969 Gitte <u>et al.</u> , 1978 Barbour <u>et al.</u> , 1991
Oxaloacetic acid	Sugarmaple	Smith, 1969
Tartaric acid and Oxalic acid	Sorghum Mustard	Bhuvaneswari and Rao, 1957 Bhuvaneswari and Rao, 1957
Lactic acid	Barley Cotton Peas	Kovacs, 1971 Kovacs, 1971 Kovacs, 1971
Glycolic acid	Barley Lentil	Kovacs, 1971 Rai, 1987
Gluconic acid Succinic acid Fumaric acid	Cicer Lentil	Gitte <u>et al.</u> , 1978 Rai, 1987

2.4.4 Fatty acids and sterols

Fatty acids and sterols which are important in the activities of some microorganisms in the rhizosphere have also been reported in root exudates. Palmitic, stearic, oleic, linoleic acids have been found in bean (Papavizas and Kovacs, 1972) and cholesterol, campesterol, stigmasterol and sitosterol were found in peanut root exudates (Thompson and Hale, 1983).

2.4.5 Vitamins

The vitamins needed to satisfy the requirements of certain microorganisms in the rhizosphere may be provided through exudation from plant roots, which may provide advantage for competitive colonization of these microorganisms. West (1939) reported that thiamine and biotin were present in the root exudate of flax. Rovira and Harris (1961) determined the B-group vitamins in leucerne, white clover, pea and tomato root exudates and found that biotin was excreted in significant quantities while all other B-vitamins were present in traces except pyridoxine which was absent. Sulochana (1962) reported the presence of thiamine, choline and inositol in the root exudate of healthy cotton plants.

2.4.6 Enzymes

Knudson and Smith (1919) reported amylase and invertase in corn and peas, respectively while phosphatase activity was found in sloughed off root cap cells of corn and tomato (Rogers et al., 1942). Cellulase was found in the root exudates of clover (Bonish, 1973) and Miskovic et al. (1977) reported higher dehydrogenase activity in the rhizosphere of corn than in the root-free soil.

2.4.7 Flavonoids

Traces of flavonoids were first reported in wheat (Lundegardh and Stenlind, 1944). Flavonoids like 7,4-dihydroxy flavone, 7,3,4-trihydroxy flavone, 7,4-dihydroxy-3'methoxy flavone were found in lentil root exudates while soybean root exudates contained coumestrol, diadzein (D'Arcy Lameta, 1986) and genistein (Kosslak, et al., 1987). Dihydroxy-2-methoxy chalcone, 7,4-dihydroxy flavone, liquiritigenin and formononetin were found in alfalfa root exudates (Maxwell et al., 1989; Hartwig et al., 1990a; 1990b; Maxwell and Phillips, 1990; Hartwig et al., 1991) while bean root exudates contained genistein, eriodictyol and naringenin (Hungria et al., 1991a, 1991b). Hydroxybenzoic acid, gentisic acid, protocatechuic acid, vanillic and salicylic acid were identified in the pea root exudate by Firnin et al. (1986) and Kovacs (1971). Clover root exudate was found

to contain 7,4-dihydroxy flavone, geraldone, 4-hydroxy-7-methoxy flavone (Redmond et al., 1986; Firnin et al., 1986), formononetin and umbelliferone (Djordjivic et al., 1987) while the flavonoid components in the vetch root exudates were identified as tetrahydroxy-4'methoxy flavonone, dehydroxy flavonone, dehydroxy-4-methoxy flavonone, trihydroxy chalcone, naringenin and liquiretigenin (Zaat et al., 1989; Recourt et al., 1991).

2.4.8 Miscellaneous compounds

Nucleic acid derivatives were reported by Fries and Forman (1951) in the root exudates of peas. Various plant growth promoting substances have also been reported in the root exudates (Linder et al., 1957; Mitchell et al., 1959; Reddy et al., 1989).

2.5 SITES OF EXUDATION

Knowledge of the sites of excretion of organic materials by the roots may allow for greater understanding of the speed and nature of development of rhizosphere and root surface microflora of young seedlings. The nature of organic constituents vary at different positions along the root, and as many microbial species show specific requirements, it is conceivable that the diversity and the nature of microorganisms associated with the rhizosphere will also vary along the length of the root (VanEgeraat, 1979). The physiologi-

cal gradients generated along the root is thought to be involved in chemotaxis, electrotaxis and pH taxis of microorganisms to specific regions on the root (Lynch, 1990).

Lundegardh and Stenlid (1944) found that the greater part of the nucleotides secreted by excised pea roots came from the tip region. Schroth and Snyder (1961) showed that the exudation of sugars and amino acids was most abundant from germinating bean seeds and root tips. Pearson and Parkinson (1961) demonstrated that the meristematic zone immediately behind the root cap is the major source of exudates in broad bean. In wheat, the zone behind the root tips is the site of maximum exudation with the next greatest quantity being lost from the zone of elongation (McDougall and Rovira, 1970). VanEgeraat (1978) reported that in peas, an unusual amino acid beta-alanine is lost only from the root tips of peas but not from other areas of the root, while homoserine was the main component released from the wounds formed on the main root due to formation of lateral root (VanEgeraat, 1975). In oats grown in axenic culture, rhizodeposition of C-compounds occurred only at root tips (Trofymow et al., 1987). A significant amount of exudation have also been noted to occur from older roots in sunflower (Frenzel, 1960).

2.6 FACTORS AFFECTING ROOT EXUDATION

Various factors affect the kind and the quality of substances released by the roots into the rhizosphere.

2.6.1 Plant species and development

Differences in the composition and quality of root exudates are thought to be greater among plants that are not closely related phylogenetically. Rovira (1956) found both quantitative and qualitative differences in the exudates of peas and oats grown aseptically, as peas released 22 amino compounds over a 21 day period while oats released only 14 amino compounds. Amino acid exudation by Phalaris and tomato exceeded that of clover growing in nutrient solution (Rovira, 1959). Pea plants grown aseptically in distilled water produced more of B-group vitamins than did leucerne, white clover, canary grass and tomato (Rovira and Harris, 1961). Young wheat and barley plants released different kinds and amounts of amino acids and carbohydrates (Vancura, 1964). Differential exudation among plants has also been reported by Vancura and Hovadik (1965) between red pepper and tomato plants grown hydroponically in gravel.

Nature of exudate also varies with plant age and development. As the plant ages both the quantity and composition of organic compounds change (Hale et al., 1978; Vancura et

al., 1977)). Pepper and tomato plants were found to release higher concentration of exudates especially carbohydrates like fructose and some oligosaccharides during their initial growth phase than during the later stages of growth (Vancura and Hovadik, 1965). Analysis of exudates showed the diverse nature of carbohydrates, amino acids and organic acids released from zero to three week old sugarmaple when compared to a 55 year old tree (Smith, 1970). Hamlen (1972) reported a decrease in the total carbohydrates released from alfalfa plants with increasing plant age over a period of 16 weeks when grown in gnotobiotic culture. The amount of sugars exuded by the young and mature blue gramma plants also varied with the phenological stage of plants (Bokhari et al., 1979).

Exudates vary in quantity and chemical composition according to whether they are released from germinating seeds or from developing seedlings. Seed exudation of barley and wheat yielded a lower spectrum of sugars than the exudation of seedlings (Vancura and Hanzlikova, 1972).

2.6.2 Temperature and light

Rate of exudation generally increases for most plants with an increase in temperature above that which is optimal for plant growth, while some species release greater quantities at low temperatures (Hale et al., 1971; Hale and Moore,

1979). High temperatures of 31°C induced increase in amino acids exuded by tomato and clover roots in axenic nutrient solution (Rovira, 1959). Hussain and Mckeen (1963) reported that amino acid exudation from straw berry roots is favored at 5-10°C. Cotton and bean exudation was found to increase at 37°C while that of peas decreased (Schroth et al., 1966). Exudation by maize and cucumber increased when subjected to a period of low temperature (Vancura, 1967). Hayman (1970) reported that carbohydrates from cotton seeds exuded faster and in larger amounts at 12-18°C than at 24-36°C.

Light intensity by affecting photosynthesis influences both the qualitative and quantitative nature of root exudates. Exudation of serine, glutamic acid and alpha-alanine by clover roots in nutrient solution decreased when day light intensity was reduced by 40 per cent (Rovira, 1959). Tomato roots exuded less aspartic acid, glutamic acid, phenyl alanine and leucine under reduced light whereas serine and asparagine increased, while clover roots were found to exude more biotin at higher light intensity (Rovira and Harris, 1961).

2.6.3 Soil moisture and atmosphere

Stress created by low soil moisture in many cases increases exudation of various compounds. The seedlings of pea, soybean, wheat, barley and tomato exuded more amino

acids when dried to wilting point than when they are kept constantly moist (Katznelson, 1955). Increased exudation due to decrease in soil moisture was also reported by Martin (1977) in wheat. A decrease in root exudation with decrease in soil moisture was reported in Pinus seedlings (Reid, 1974).

Gas exchange which is closely related with soil type and moisture, affects both root development and exudation. Roots of young corn and sunflower when subjected to a period of anaerobiosis exuded more amounts of alanine, proline, glutamic acid, serine, threonine and aspartic acid when compared to normal plants (Grineva, 1962). Peanut plants in nutrient solution when subjected to varying O_2 and CO_2 tensions responded differently by exuding greater amounts of galactose and dihydroxy acetone under aerobic conditions (Rittenhouse and Hale, 1971).

2.6.4 Plant nutrition

Nutrient uptake by root systems are logically related to root exudation. Calcium is known to affect membrane permeability which may affect root exudation (Vancura, 1967). Bowen (1969) found that phosphate deficiency leads to increased exudation as opposed to low exudation by nitrogen deficient plants. Low levels of calcium increased the root-cell membrane permeability of peanut resulting in a

quantitative increase in exudation of sugars from peanut roots (Shay and Hale, 1973).

2.6.5 Plant injury factors

The amount of substances liberated from the roots varies widely according to the conditions in which the root system develops. Boulter et al. (1966) found that greater amounts of various amino acids were exuded by pea seedlings in quartz sand than in a culture solution. Barber and Gunn (1974) showed that the roots of barley and maize grown in nutrient solution with glass beads released more amino acids and carbohydrates than the roots of the plants grown in liquid culture alone.

2.6.6 Foliar spray

Foliar application of fertilizers and pesticides can also affect the quantity and composition of root exudates. Davy and Papavizas (1961) observed suppression of gram negative bacteria when sprayed with labeled streptomycin. Organic compounds applied to foliage have been detected in the rooting solution in the rhizosphere (Linder et al., 1964; Reid and Hurtt, 1970). Hormones applied to leaves can also influence the rhizosphere microflora as was seen by the increase in the rhizosphere microflora of Phaseolus due to the foliar spray of indole acetic acid (Singh, 1982).

2.6.7 Microbial effects

The presence of microorganisms in the rhizosphere generally increases root exudation. Barber and Martin (1976) found that, in wheat and barley plants under sterile conditions, about 5-10 per cent of the photosynthetically fixed carbon was released by the roots, compared with 12-18 per cent released from roots in non-sterile soil. Exudates from nodulated root systems are some times different from others in systems without Rhizobium nodules. Inoculation of alfalfa with Rhizobium resulted in an increase in the non-reducing sugars in the root exudate while reducing sugars and total phenols decreased (Hale and Moore, 1979). Prikryl and Vancura (1980) observed that wheat plants cultivated in the presence of Pseudomonas putida released upto twice the amount of exudate released by axenically cultured plants. Agropyron roots, grown in the presence of microorganisms were also reported to release approximately 2-6 times the carbon released under sterile conditions (Biondini et al., 1988).

2.7 INFLUENCE OF ROOT EXUDATES ON RHIZOSPHERE POPULATION

The quantitative and qualitative value of microbial biomass are related either directly or indirectly to the root exudates. Root exudates play an important role in regulating interactions between plant host and pathogens in

the rhizosphere by stimulating and inhibiting spore generation, mycelial growth, chemotaxis and binding to the root. Regions where exudates and root derived organic matter are available also produce favorable microenvironment for the beneficial microorganisms. Vancura and Macura (1961) found that the exudates of wheat and barley showed stimulating effect on Azotobacter chroococcum.

Similarly, though the rhizobia exhibit marked specificity for the hosts which they invade and nodulate, the plant is not a passive partner in the initiation of nodulation, and it tightly regulates susceptibility to infection and subsequent development of infective nodules (Hedges and Messens, 1990). The plant controls nodule formation at the biochemical, genetic, physiological and ultrastructural levels of the symbiotic association (Caetano-Anolles and Gresshoff, 1991). Since, rhizobia nodulate only legumes, it is thought that rhizobia are selectively favored by substances excreted into the rhizosphere by the host plant (Schmidt, 1979) which affect the rhizobial processes like chemotaxis, growth and nod gene induction.

2.7.1 Chemotaxis

To initiate an interaction with the appropriate host plant the microorganisms in question must somehow arrive at the root surface as a component of the rhizosphere popula-

tion (Schmidt, 1979). How the rhizobia arrive at the root surface has been a long studied subject. It has been suggested that rhizobia migrate to root through chemotactic responses (Currier and Strobel, 1976). Bacterial chemotaxis towards plant root exudates is an important mechanism of colonization by beneficial and harmful microorganisms (Barber and Cher, 1987). The wide spread occurrence of flagellate bacteria (Carlile, 1980) and the copious exudation into the root zone by legume roots of a wide variety of compounds (Beringer et al., 1979) suggested that chemotaxis may have a role in the establishment of the legume-Rhizobium symbiosis. Rhizobia like other bacteria move towards certain compounds often potential nutrients (Sprent and Sprent, 1990). The production of an attractant may be the first signal from a plant to a potential rhizosphere colonist (Lynch, 1990). The attractant may contain specific information concerning the quality of the host.

CHEMOTAXIS OF RHIZOBIA

It has been widely reported that rhizobia migrate towards root exudates. Chemotaxis as a mechanism for attraction of rhizobia to the legume rhizosphere was first reported by Currier and Strobel (1976). Rhizobia have also been reported to undergo taxis towards increasing concentrations of a glycoprotein exuded by birdsfoot trefoil (Currier

and Strobel, 1977). Simple compounds present in the legume root exudate may attract the rhizobia and aid in their rhizosphere accumulation and, thus in the nodulation process (Hunter and Fahring, 1980). A number of compounds which serve as attractants for diverse strains of rhizobia have been identified. Amino acids and sugars have been the most studied, compounds since they have been identified in the legume root exudates.

CHEMOTAXIS TOWARDS SUGARS

Rhizobium leguminosarum

Rhizobium leguminosarum WIII 63 showed chemotaxis to L-arabinose, D-ribose, D-glucose, D-fructose, mannitol, sorbitol, dulcitol, lactose and cellulose, but it was not chemotactic to either sucrose or trehalose (Bowra and Dilworth, 1981). Gaworzewska and Carlile (1982) reported that R. leguminosarum nodulating peas showed positive chemotaxis towards arabinose, fructose, galactose, glucose, lactose, maltose, mannitol, ribose and xylose at 10^{-3} to 10^{-4} M concentration, while glucose, raffinose, trehalose and sucrose failed to elicit a chemotactic response. Rhizobium leguminosarum which nodulates lentil showed chemotactic responses to glucose, xylose, fructose and mannitol (Rai, 1987). Xylose (10^{-4} M), sucrose (10^{-6} M) and raffinose (10^{-6} M) were among the strong chemoattractants for R.

leguminosarum biovar phaseoli RP 80012 while glucose, galactose and maltose produced little or no detectable response (Aguilar et al., 1988).

Bradyrhizobium japonicum

Hunter and Fahring (1980) found arabinose to be an attractant for R. japonicum but other sugars elicited no response. None of the sugars tested (mannitol, arabinose, galactose, sucrose and glucose) were found to be significant attractants for B. japonicum (Barbour et al., 1991).

Rhizobium meliloti

Rhizobium meliloti has relatively weak responses to sugars as only gluconate served as an attractant among the various sugars tested (Burg et al., 1982). Similarly, carbohydrates especially glucose and mannitol were also reported as poor attractants of R. meliloti (Gotz et al., 1982) while L-arabinose, L-arabitol, D-fructose, D-galactose, glycerol and maltose were found to elicit weak response. Unlike others, Malek (1989) reported positive chemotaxis towards sugars by R. meliloti.

Rhizobium lupini

Rhizobium lupini was attracted to D-glucose, D-mannitol, L-arabinose and D-galactose but showed little response towards L-arabitol, D-fructose, glycerol and mal-

tose (Gotz et al., 1982) though it exhibited greater chemotaxis towards sugars than amino acids.

Cicer Rhizobium

Gitte et al. (1978) reported that Rhizobium sp. specific to chickpea showed maximum chemotaxis towards glucose found in the root exudate of chickpea whereas xylose, galactose, ribose and arabinose elicited little response from the Rhizobium. A similar chemotactic trend was also observed by Kush and Dadarwal (1981) who reported that cicer Rhizobium showed no chemotaxis towards the sugars arabinose, ribose, xylose and mannose while glucose and galactose elicited a chemotactic response.

CHEMOTAXIS TOWARDS AMINO ACIDS

Rhizobium leguminosarum

Rhizobium leguminosarum showed significant chemotaxis towards all the 30 amino acids tested at 10^{-5} M dilutions while arginine, glycine and lysine elicited significant response to 10^{-6} M dilution also (Gaworzewska and Carlile, 1982). Rhizobium leguminosarum nodulating lentil was found to be chemotactic towards histidine, serine, valine and isoleucine while another strain showed chemotaxis to all amino acids (Rai, 1987).

Bradyrhizobium japonicum

Hunter and Fahring (1980) observed migration of B. japonicum in response to canaxamine but not with 15 other amino acids tested. Only glutamate and aspartate were strong attractants to B. japonicum among the 14 amino acids tested (Barbour et al., 1991).

Rhizobium meliloti

Glucose, leucine, proline, lysine and arginine were good attractants while aspartate and glutamate were poor attractants of R. meliloti (Gotz et al., 1982). Amino acids with an acidic or basic character like aspartate, arginine, cysteine, histidine, leucine and methionine were stronger attractants than unipolar amino acids (Burg et al., 1982).

Rhizobium lupini

Rhizobium lupini was attracted to all L-amino acids with the notable exception of leucine which elicited no positive response. Glycine, isoleucine, homoserine, serine, threonine, cysteine, glutamine, glutamate were weaker attractants (Gotz et al., 1982).

Cicer Rhizobium

Gitte et al. (1978) reported that cicer Rhizobium strain exhibited maximum attraction towards histidine. The

order of attraction from highest to lowest was as follows: Histidine, serine, lysine, glycine, arginine, aspartic acid, leucine, isoleucine and lastly cysteine. Cicer Rhizobium showed chemotaxis towards asparagine, tyrosine and tryptophan at 10 uM concentration and towards glutamine at 1.0 uM whereas glutamine, isoleucine, glucosamine, serine and homoserine did not elicit any chemotactic response (Kush and Dadarwal, 1981).

CHEMOTAXIS TOWARDS ORGANIC ACIDS

Rhizobium leguminosarum was found to be chemotactic towards citric, malic gluconic and succinic acids found in pea root exudates (Gaworzewska and Carlile, 1982). Succinic acid was found to be an attractant for R. leguminosarum strain nodulating lentil while citric, gluconic and fumaric acids were found to be main attractants for another R. leguminosarum strain which also nodulates lentil (Rai, 1987).

Bradyrhizobium japonicum showed chemotaxis towards succinate, malonate, malate, L-beta-glutarate and fumarate but not towards pyruvate and citrate (Barbour et al., 1991) while Parke et al. (1985) reported that B. japonicum showed positive chemotaxis only towards succinate and beta-keto-adipate.

CHEMOTAXIS TOWARDS PHENOLIC COMPOUNDS

Slow growing B. japonicum showed positive chemotaxis to shikimate, quinate, protocatachuate and vanillate while R. trifolii exhibited positive chemotaxis to shikimate with quinate, protocatachuate and vanillate being weak attractants (Parke et al., 1985).

CHEMOTAXIS TOWARDS FLAVONOIDS

For R. leguminosarum bv. phaseoli, 10 uM apigenin and 10 uM luteolin were strong attractants but naringenin had no effect (Aguilar et al., 1988). Rhizobium leguminosarum bv. viciae moved towards 5 uM naringenin, 10 uM kaempferol and 50 uM apigenin but hesperitin elicited no chemotaxis (Armitage et al., 1988).

Rhizobium meliloti gave positive chemotactic response to 10 mM luteolin but it did not respond to either apigenin or naringenin (Caetano-Anolles et al., 1988). It was also reported that R. meliloti is chemotactic to 7,4 dihydroxy flavone (Phillips, 1992).

For B. japonicum the isoflavones genestein and diadzein which induce gene transcriptions were found to be very weak attractants (Barbour et al., 1991). Kape et al. (1991) also reported that these compounds as well as other isoflavones were not chemoattractants to B. japonicum.

It was also observed that rhizobia show chemotaxis to plant root exudates both legumes and non-legumes, though they show differential response in that they are attracted to the root exudates of some plants and not towards others (Currier and Strobel, 1976). Gaworzewska and Carlile (1982) have also reported positive chemotaxis of all the rhizobia tested (R. leguminosarum, R. trifolii and R. phaseoli) towards root exudates of clover, broad bean, mustard and maize.

As the chemotactic response of rhizobia is found to be non-specific with rhizobia migrating to exudates of a wide array of legumes and non-legumes it has been suggested that chemotaxis plays only a minor role in the migration of rhizobia to the roots. But still there are a few reports which describes enhanced nodulation by motile rhizobia over non-motile mutants (Hunter and Fahring, 1980; Soby and Bergman, 1983; Mellor et al., 1987; Catlow et al., 1990).

2.7.2 Growth and multiplication of rhizobia in the rhizosphere

Legumes support considerably larger rhizosphere populations than non-leguminous plants (Katznelson, 1965) and legumes usually induce a greater proliferation of bacteria than is observed with non-legume plants (Rouatt and Katznel-

son, 1961). Though it has been suggested that successful nodulation of legume root is preceded by proliferation of an appropriate strain of Rhizobium in the rhizosphere of the host, rhizobia are found to be good rhizosphere organisms for both legumes and non-legumes.

GROWTH OF RHIZOBIA IN THE NON-LEGUME RHIZOSPHERE

Rovira (1961) found enhancement of R. trifolii in the rhizosphere of the grass Paspalum dilatatum. Rhizosphere effect was also apparent for R. trifolii in the rhizosphere of upland rice, Sudan grass, rape seed and tomato (Tuzimura and Watanabe, 1962). Rhizobium japonicum was found to be stimulated in the rhizosphere of corn and wheat (Reyes and Schmidt, 1979) and oats (Moawad et al., 1984). It was also reported that root extracts of non-leguminous plants like Ambrosia, Aristida, Bromus, Digitaria and Helianthus strongly inhibited Rhizobium (Rice, 1971a, b; Murthy and Nagodra, 1977). Hiltbold et al. (1985) found that the populations of B. japonicum in a cotton-corn-soybean rotation decreased from 10^6 cells per gram soil during soybean cultivation to 10^2 per gram of soil after the cultivation of cotton or corn. Kucey and Haynes (1989) also found that the population of R. leguminosarum bv. phaseoli and bv. viciae were significantly lower in the field after bean or pea rotation with non-legumes like barley, wheat and sugarbeet. In a 22 year study of the fields following corn-soybean-oats-alfalfa

rotation it was found that B. japonicum was more persistent during crop rotations than R. meliloti (Triplett et al., 1993).

RHIZOBIA IN THE NON-HOST LEGUME RHIZOSPHERE

Non-nodulating soybean roots were found to exude compounds which altered the morphology of Rhizobium and prevented nodulation of the nodulating soybean line (Elkan, 1961). Root extracts of non-nodulating mutants of Cassia fistula, C. occidentalis and Leucaena leucocephala were inhibitory to several strains of rhizobia (Rao et al., 1973).

Rhizobia were also found to multiply in non-host legume rhizosphere as was reported by Robinson (1967) who found that R. trifolii which nodulates clover could also colonize alfalfa and pea. Rhizobium japonicum was slightly stimulated in the rhizosphere of bean whereas R. phaseoli strains responded well to the rhizosphere of soybean (Robert and Schmidt, 1983). Both R. leguminosarum and R. japonicum were found at higher densities in the rhizosphere of peas than in those of soybeans at early stages (1-2 d) whereas the reverse was true in the later (3-3 d) stages (Bohloul et al., 1984).

RHIZOBIA IN THE HOST LEGUME RHIZOSPHERE

Purchase and Nutman (1957) observed that there was a rise in the abundance of R. trifolii when it was introduced into the solution supporting its homologous host as opposed to the lower population in the absence of T. pratense. Chailakbyan and Megrabyan (1958) reported that root exudates of legumes contain a factor which selectively stimulates the homologous rhizobia. Rovira (1961) found that R. trifolii was stimulated to a greater extent than other microorganisms in the clover rhizosphere and also the stimulus was observed as far away as 10-20 mm from the roots unlike in the rhizosphere of Paspalum. Higher counts of clover rhizobia, R. trifolii were found in the rhizosphere of host clover rhizobia, R. trifolii were found in the rhizosphere of host clover than in the non-host legumes (common vetch, soybean and peanut) and in the non-leguminous (rice, Sudan grass, rape seed and wheat) rhizosphere (Tuzimura and Watanabe, 1962). Rhizobium strain nodulating Lotus corniculatus multiplied better in the root environs of the host plant when compared to that of the control without host plants (Peters and Alexander, 1966). Robinson (1967) found that the number of R. trifolii tended to be slightly higher in the rhizosphere of T. subterranean than in those of Medicago sativa and Poa australis while R. meliloti was very specifically influenced by M. sativa. Rhizobia are known to thrive

in soils planted with legumes and their populations fall if they are replaced by other plants (Parker et al., 1977). Rhizobium japonicum response in the rhizosphere was only slightly less for wheat than for soybeans whereas comparably slight rhizosphere stimulation was observed with corn (Reyes and Schmidt, 1979) and oats (Moawad et al., 1984). An excellent geographical correlation between growth of a variety of legumes and the distribution of rhizobia capable of nodulation, suggests that the rhizobia are favored in the presence of suitable host plants (Lie et al., 1987).

Homoserine, an unusual amino acid exuded by the pea roots, was found to selectively favor the growth of R. leguminosarum which nodulated peas (VanEgeraat, 1975). Low micromolecular concentration of flavonoids have also been reported to stimulate the growth rate of rhizobia. Bradyrhizobium japonicum grew faster with 1 uM diadzein found in the soybean seedling extracts (D'Arcy-Lameta and Jay, 1987). Rhizobium meliloti also showed increased growth rate when 2 uM luteolin and quercetin found in the alfalfa seed exudates were added to a defined medium (Hartwig et al., 1991).

2.7.3 Nod gene induction

Root exudes substances which induce physiological processes necessary for nodulation. Soybean root exudate

has been shown to enable the development of biochemically specific lectin receptors in R. japonicum strains which do not show the capability when grown in a synthetic salt medium (Bhuvaneswari and Bauer, 1978). Proteins found in the clover root exudates modified surface polysaccharides of Rhizobium cells favoring their attachment to root hairs (Dazzo, 1980). Odunfa and Werner (1981) reported that when a complete nitrogenase inducing medium was supplied with cowpea or soybean root exudate, the nitrogenase activity increased. A delay in the nodule initiation by Bradyrhizobium strains can be reversed by pretreating these strains in host root exudates (Bhagwat and Thomas, 1980) as the root exudate induces structural modification in the capsular polysaccharide of Bradyrhizobium (Bhagwat and Thomas, 1984). The root exudates obtained from young seedlings of Cicer arietinum promoted the nitrogenase activity of the chickpea rhizobia (Gupta et al., 1984). Preincubation of R. meliloti with dialyzed alfalfa root exudate was found to markedly increase early adsorption of the rhizobia to alfalfa roots. Dihydroxy flavone from the seedling extract of white clover has been identified as an inducer for R. leguminosarum bv. trifolii (Redmond et al., 1986) while genestein and diadzein found in the soybean seedling extract induced the nod genes in B. japonicum and R. friedii (Kosslak et al., 1987; Sadow-sky et al., 1988).

2.8 SEED EXUDATION

The region around the germinating seed has received less attention than the rhizosphere but its colonization by microorganisms is generally the predisposing determinant of rhizosphere colonization, as the adaptation of a microorganism to a developing rhizosphere may begin as early as the seed germination by the plant partner. The initial exudation of the plant is derived from the seeds. As the seed imbibes it immediately losses carbon in the form of amino acids, sugars, aliphatic acids, phenols and gases into the surrounding environment. This leaching from the seeds is a major source of nutrients for the microflora around the seeds.

Bean seed leacheate was found to contain the sugars, glucose, sucrose, maltose, fructose and amino acids like asparagine (Schroth and Snyder, 1961). A range of 24 amino acids and sugars like alanine, aspartic acid, glycine, maltose, galactose, glucose and fructose were detected in the seed exudate of maize (Vancura, 1967) while 14 amino acids, 4 sugars and 3 organic acids were detected in Pinus resinosa (Agnihotri and Vaartaja, 1968). Vancura and Hanzlikova (1972) compared the composition of seed leacheate and seedling exudates and reported that the spectrum of amino acids, sugars and organic acids exuded differed widely

in barley, wheat, cucumber and bean. Sugars, amino acids and organic acids were also exuded by seeds of pea, barley, cotton, groundnut, lupin and maize (Kovacs, 1971; Wadha et al., 1989; Berestetskii and Kravchenko, 1981). The loss of water soluble exudates was directly correlated to the seed size (Vancura and Hanzlikova, 1972; Naim et al., 1976; Vancura and Stotzky, 1976; Nelson et al., 1986).

Various flavonoid compounds have also been detected in the seed leacheates. Alfalfa seed leacheates contained luteolin, chrysoeriol and quercetin (Hartwig et al., 1989; Hartwig et al., 1990a) while bean seed leacheate contained delphinidin, myricetin, quercetin and kaempferol (Hungria et al., 1991b). Pea seed rinse was found to contain the flavonoids apigenin-7-O-glucoside and eriodictyol (Firmin et al., 1986).

As the dry seeds imbibe and germinate they also release volatile and gaseous compounds like ethanol, methanol, formaldehyde, acetaldehyde, formic acid, ethylene and propylene. Germinating seeds of bean, corn and cotton released larger quantities of volatiles than those of cucumber, cabbage and squash (Vancura and Stotzky, 1976). Germinating seeds of several plant species evolved oxidizable volatiles including aliphatic aldehydes and larger seeds were found to evolve greater quantities of volatiles (Stotzky and Schenck, 1976). Gorecki et al. (1985) observed that imbibing and

germinating pea seeds produced ethanol, acetaldehyde and methanol.

Reddy et al. (1977) revealed that germinating groundnut seed exudate contained chromogenic, caffeic, coumeric, hydrobenzoic, ferulic and vanillic acids. Kovacs (1971) found traces of hydrobenzoic acid in the germinating pea and barley seed exudates while coumeric acid was the main constituent of the germinating cotton seed exudate.

2.8.1 Effect of seed leachate and germinating seed exudate on soil microflora

Seed exudates are an important source of nutrients to the soil microorganisms. Seed exudates act as stimulants for spore germination of many plant pathogenic fungi (Agnihotri and Vaartaja, 1968; Schlub and Schmitthenner, 1978; Weinhold et al., 1972). The volatiles in the seed exudates were also found to increase the growth of many other pathogenic microorganisms like Pseudomonas, Bacillus, Erwinia, Agrobacterium, Mucor and Fusarium (Schenck and Stotzky, 1975).

EFFECT OF THE SEED LEACHATE AND GERMINATING SEED EXUDATE ON RHIZOBIA

Germinating seed exudates of plants especially the legumes have been found to be inhibitory to rhizobia.

Rhizobium trifolii was inhibited by the seeds of subterranean clover (Thompson, 1960) while Bowen (1961) found that the seed leacheates of Centrosema pubescens and T. subterranean were inhibitory to several Rhizobium strains. Seed diffusates of pea, Bengal gram, green gram, soybean, Lucerne, berseem, cowpea, black gram and red gram were found to be toxic towards rhizobia (Dadarwal and Sen, 1973) as were the seed leacheates of lablab, clusterbean and horsegram (Kandaswamy et al., 1974). Materon and Weaver (1984) observed the toxicity of clover seed to R. trifolii while lima bean, lupin, soybean and sesbania seed leacheates inhibited their respective rhizobia i.e., Rhizobium sp. nodulating lima bean, R. lupini, B. japonicum and Azorhizobium (Ali and Abdel-Moneni, 1986; Antipchuk et al., 1990; Ali and Loynachan, 1990; Balasubramanian et al., 1992).

The toxic constituents were identified as either flavonoids (Fortell et al., 1964), tannic acid (Young and Paterson, 1980) or phenols (Kandaswamy and Prasad, 1978).

Volatiles from various species of germinating seeds were found to increase the growth of Rhizobium japonicum (Schenck and Stotzky, 1975).

Carbohydrate pinitol leached from soybean during inhibition is thought to have a role in Rhizobium infection

(Nordin, 1984). Bradyrhizobium japonicum was also found to be strongly chemotactic towards soybean seed exudates (Barbour et al., 1991).

MATERIAL AND METHODS

III. MATERIAL AND METHODS

Experiments to determine the effect of root exudate compounds of legumes and non-legumes on rhizobia were conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences (UAS), G.K.V.K. Campus, Bangalore.

Four strains of rhizobia maintained in the Department of Agricultural Microbiology, UAS, Bangalore were used in the study: a chickpea strain (IC 59), a cowpea strain (P 132), a groundnut strain (TAL 1000) and a soybean strain (TAL 102). These cultures were maintained on slants of yeast extract mannitol agar medium containing (in g/L) mannitol, 10; NaCl, 0.1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; K_2HPO_4 , 0.5; and yeast extract, 0.5. The plant species used in the study were as follows: (a) Legumes: chickpea (Cicer arietinum L.) cv. Annageri, cowpea (Vigna unguiculata) cv. C-152, groundnut (Arachis hypogaea L.) cv. TMV-2, soybean (Glycine max (L.) Merr.) cv. KHSB-2 and (b) Non-legumes: corn (Zea mays L.) cv. Deccan-103, ragi (Eleusine coracana Gaertn.) cv. MR-1, cotton (Gossypium hirsutum) cv. hybrid Varalakshmi and sunflower (Helianthus annus) cv. BSH-1. The other chickpea varieties - the high nodulating ICC 4948, the medium nodulating ICC 4948 and the low nodulating ICC 4948 were obtained from Dr. O.P. Rupela, at ICRISAT, Hyderabad, India.

3.1 SURFACE STERILIZATION OF SEEDS

Seeds were surface sterilized by immersion for 5 sec in 75 per cent ethanol, followed by surface sterilization for 5 min in 0.2 per cent HgCl_2 acidified with 0.5 per cent HCl . Seeds were washed six times in sterile distilled water and then used for the study (Caetano-Anolles and Favelukes, 1986).

3.2 COLLECTION OF SEED LEACHEATES

Ten g of surface sterilized seeds were soaked in 100 ml of sterile distilled water for 18 h and the diffusate was subsequently decanted and used as seed leacheates.

3.3 COLLECTION OF ROOT EXUDATES

Surface sterilized seeds were germinated in dark at 30°C on water agar for 48-72 h. Two germinated seeds were transferred aseptically to sterile culture tubes (20.0 x 2.5 cm) containing 10.0 ml of distilled water with 0.5 mM CaSO_4 as the root exudate collecting solution. The germinated seeds were supported on the bent loop of a glass rod placed inside the culture tube (Plate 1). The loop being 0.5 cm above the level of distilled water allowed only the roots to extend into the exudate collecting solution. The plants were grown for 10 days under fluorescent lights after which the root exudates were collected (Karthikeyan, 1992).

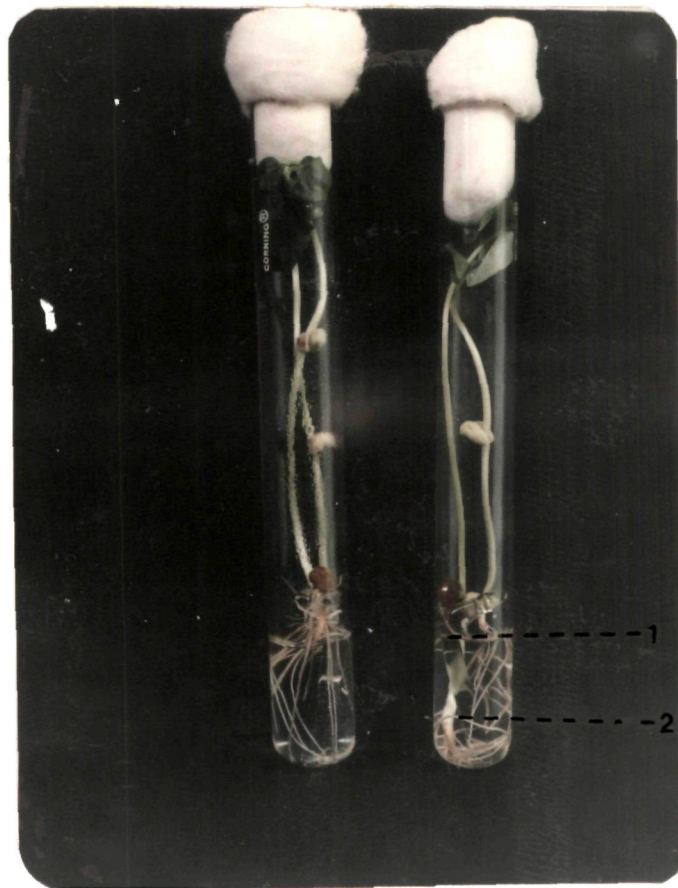


Plate 1: Collection of root exudates

1. GLASS ROD SUPPORTING GERMINATING SEEDS
2. DISTILLED WATER INTO WHICH ROOTS OF SEEDLINGS EXTEND

3.4 ESTIMATION OF RHIZOSPHERE POPULATION

The pregerminated surface sterilized seeds were transferred into culture tubes as described previously. The four rhizobial strains, IC 59, P 132, TAL 1000 and TAL 102 were grown at 30°C for 2-3 days in yeast extract mannitol broth. The cells were collected by centrifugation at 8000 g for 10 minutes at 4°C, resuspended in a sterile buffer solution containing 0.8 g K_2HPO_4 and 0.2 g KH_2PO_4 to a density of approximately 1.0×10^4 cells per ml. One ml of the suspension of each rhizobial strain was introduced into the plant culture tubes with distilled water along with germinated seed. The culture tubes with the plants and the introduced rhizobia were grown for 10 days under fluorescent lights after which rhizobial counts in the root exudate solution (rhizosphere) were made (Peters and Alexander, 1966). The contents of each tube were shaken thoroughly and the cell density was estimated by Miles and Mishra drop plate method.

Each plant species was inoculated with the four rhizobial strains and each treatment was maintained in triplicate. Controls (also in triplicate) were maintained by introducing the same inoculum into distilled water in culture tubes without plants.

3.5 BIOASSAY FOR INHIBITION BY GERMINATING SEEDS

Agar plates were prepared by uniformly adding to the surface of yeast extract mannitol agar plates one ml of the test strain at a density of 10^8 cells per ml. Three surface sterilized seeds were placed aseptically on the agar surface. Plates were then kept at 28°C and observations of the zones of inhibition was made after four days. The zone of inhibition was assessed by measuring the distance between the edges of the inhibition on either side of the seed (Ali and Loynachan, 1990).

The experiment was carried out with three replications.

3.6 CHEMOTAXIS OF RHIZOBIAL STRAINS TOWARDS SEED LEACHEATE AND SEEDLING ROOT EXUDATES

Alders capillary tube method (Alder, 1973) was used for the chemotaxis assays except that 2.5 mm x 4 mm glass test tubes were substituted for the cover slip - glass slide assembly (Bowra and Dilworth, 1981).

Freshly collected unconcentrated root exudates and seed leacheates were collected as mentioned before and were used as chemoattractants in the assay. Rhizobial suspensions were prepared by centrifuging the rhizobial cultures grown in yeast extract mannitol broth at $2500 \times g$ for 10 min and the cells were resuspended in 5.0 ml of 10.0 mM Potassium-

Phosphate buffer (pH 6.5). After further centrifugation, the rhizobia were resuspended in the 10.0 mM Potassium-Phosphate buffer (motility medium) to give approximately 1.0×10^4 cells per ml.

The chemotaxis assay was conducted as follows:

Capillary tubes (4.5 cm long with 0.2 mm internal diameter) were used for containing the attractant. One end of the capillary was sealed in a flame. The capillary was then quickly passed several times through the flame and immediately plunged open end down into a 10 ml beaker containing 1.0 ml of the attractant (seed leacheate/root exudate). As the capillary cooled, the attractant was drawn in about 1 cm. After 10 min the capillary was inserted open end first into a glass tube (2.5 mm x 4 mm) containing 0.2 ml bacterial suspension. After incubation for 1 h the capillaries were removed. It's exterior was rinsed with a thin stream of water. The sealed end was broken and the total content of the capillaries thoroughly mixed in a 9.0 ml water blank, appropriately diluted and plated on yeast extract mannitol agar. The plates were incubated for 5 days at 28°C. The number of colonies were recorded and the cell density was calculated. A control chemotaxis assay for the four rhizobial strains was conducted

simultaneously by using distilled water in the capillaries in the place of the attractant. All the treatments were maintained in triplicate.

Chemotactic response of the rhizobial strain was expressed in terms of relative responses calculated as the number of colony forming units per capillary containing the extractant divided by the number of colony forming units in the capillaries containing controls.

3.7 ESTIMATION OF AMINO ACID COMPOSITION OF THE ROOT EXUDATES AND SEED LEACHATES

The exudates collected were passed through a millipore filter (0.45 μ) and concentrated by lyophilization to about 1.0 ml per seed or per seedling and was used for the determination of amino acids in the exudates/leachates.

To the concentrated exudate/leachate, 5.0 ml of 80 per cent ethanol was added and then filtered to obtain total free amino acids. Using this extractant amino acids were determined by radial paper chromatography. Standards were prepared by dissolving individual amino acids in distilled water at a concentration of 1.0 μ g per ml. Very dilute HCl was used to dissolve the free amino acids tyrosine and phenyl alanine. Samples of exudates as well as the standard amino acids were spotted on to the chromatograph sheet and allowed to develop in a mobile phase containing n-butanol,

glacial acetic acid and water in the ratio 4:1:5. The chromatograms were then dried and sprayed with ninhydrin and allowed to dry yet again till the purple amino acid spots appeared. The spots of the amino acids were marked and then Rf values were calculated by the formula:

$$R_f = \frac{\text{distance (cm) moved by the solute from the origin}}{\text{distance (cm) moved by the solvent from the origin}}$$

The amino acids present in the sample were identified by comparing the Rf values with that of the authentic amino acids which were chromatographed simultaneously (Sadasivan and Manickam, 1992).

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of the investigation conducted to determine the effect of legume and non-legume exudates on rhizobia are presented in this chapter. The legumes: chickpea, cowpea, soybean and groundnut and the non-legumes: maize, ragi, sunflower and cotton along with four Rhizobium strains IC 59, P 132, TAL 1000 and TAL 102 were used in the study.

4.1 GROWTH OF RHIZOBIA IN THE ROOT EXUDATES OF LEGUMES AND NON-LEGUMES

Rhizosphere population of rhizobia were estimated 10 days after the inoculation of the seedlings by dilution plating technique. It was observed that within 10 days the rhizobial cell densities increased rapidly and reached a peak of 10^7 cells per ml from the initial population of 10^3 cells per ml. The inoculum introduced into distilled water without any roots yielded populations 10-fold smaller than the populations observed in the presence of roots. This shows that there is a rhizosphere population increase in the presence of root exudates (Table 4 and 5).

A difference in the response of Rhizobium strains to legumes and non-legumes was observed. The highest population of IC 59 was noted in the root exudates of high nodulating chickpea (7.6732 log cells/ml) while the lowest population was found in sunflower root exudates (7.583 log

Table 4: Growth (log number of colony forming units of rhizobia/ml)* of rhizobial strains in the root exudate of legume seedlings

Legumes	Rhizobial strains/Host legumes			
	IC 59/ Chickpea	P 132/ Cowpea	TAL 1000/ Groundnut	TAL 102/ Soybean
Chickpea (High nodulating)	7.6732	7.5873	7.5868	7.5807
Chickpea (Medium nodulating)	7.6698	7.5835	7.5835	7.5749
Chickpea (Low nodulating)	7.6666	7.5711	7.5817	7.5667
Chickpea cv. Annageri	7.6682	7.5682	7.5778	7.5652
Cowpea	7.6499	7.6506	7.6056	7.5855
Soybean	7.6401	7.5612	7.5740	7.6056
Groundnut	7.6449	7.6418	7.6115	7.5901

* The root exudate was inoculated with 1×10^3 cells of rhizobia two days after germination of seed and growth was recorded ten days after inoculation

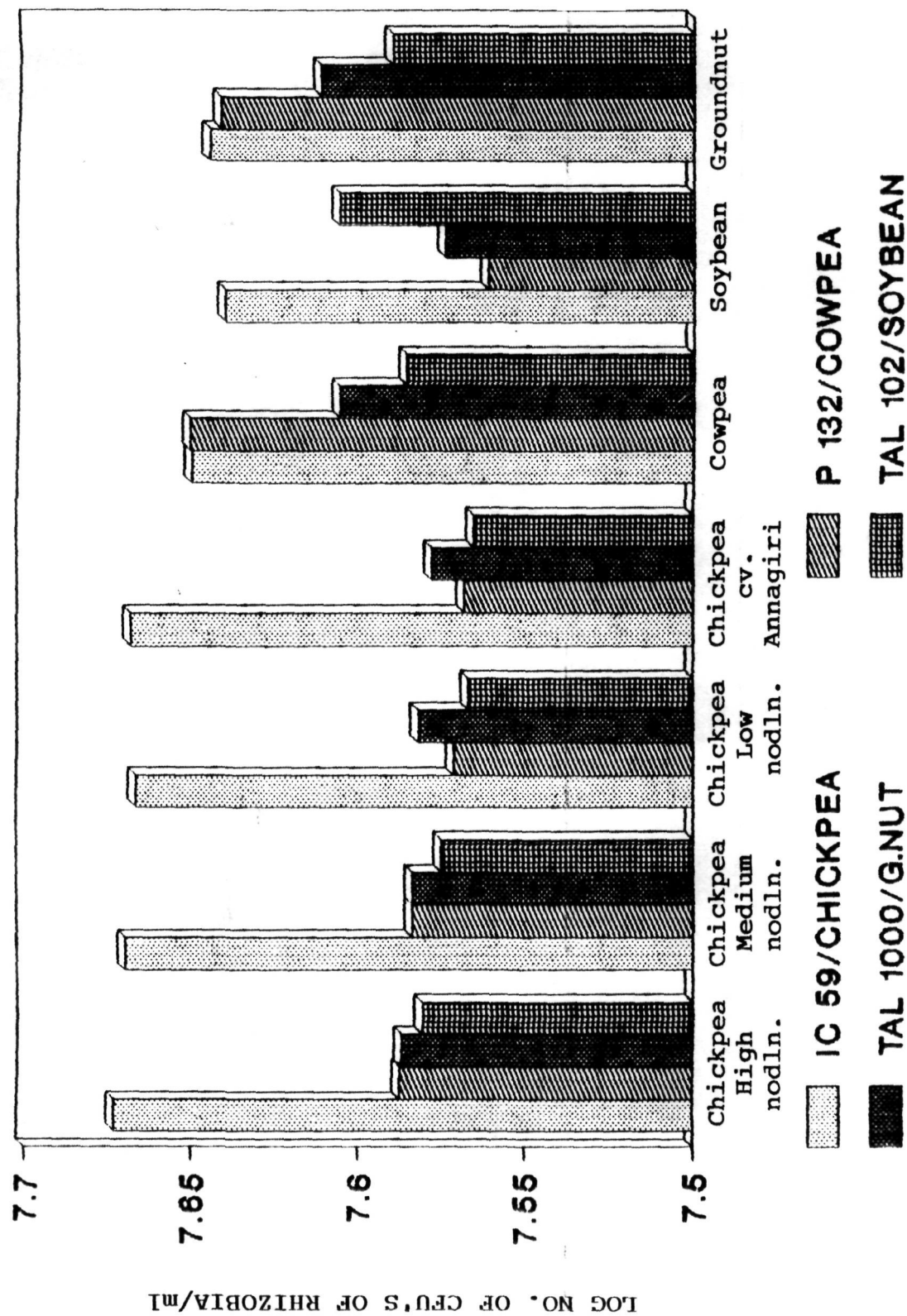


Fig. 1: GROWTH (LOG NO. OF CFU'S OF RHIZOBIA/ML) OF RHIZOBIAL STRAINS IN THE ROOT EXUDATE OF LEGUME SEEDLINGS

Table 5: Growth (log number of colony forming units of rhizobia/ml)* of rhizobial strains in the root exudate of non-legume seedlings

Non-legumes	Rhizobial strains/Host legumes			
	IC 59/ Chickpea	P 132/ Cowpea	TAL 1000/ Groundnut	TAL 102/ Soybean
Maize	7.5928	7.5481	7.5711	7.5652
Ragi	7.5873	7.5367	7.5607	7.5632
Sunflower	7.5835	7.5234	7.5325	7.5604
Cotton	7.5844	7.5267	7.5420	7.5642

* The root exudate was inoculated with 1×10^3 cells of rhizobia two days after germination of seed and growth was recorded ten days after inoculation

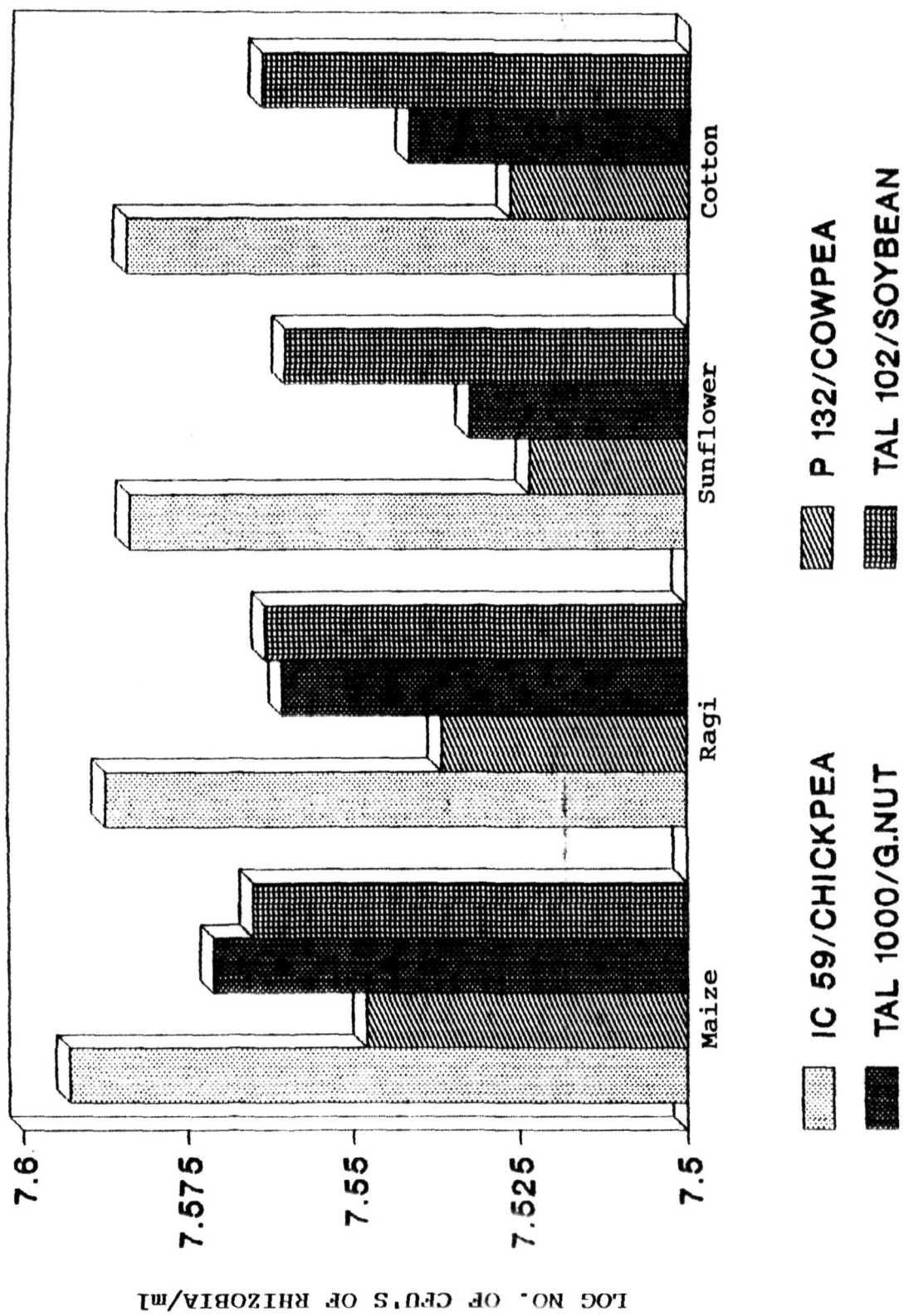


Fig. 2: GROWTH OF (LOG NO. OF CFU'S OF RHIZOBIA/ml) OF RHIZOBIA IN THE ROOT EXUDATE OF NON-LEGUME SEEDLINGS

cells/ml). Among the legumes IC 59 had higher population densities in the root exudates of chickpea varieties. There was very little difference between the population of IC 59 in the root exudates of high nodulating, medium nodulating and low nodulating chickpea isolates and chickpea cv. Annageri though the high nodulating isolate showed the highest population density. The response of IC 59 (7.6499 log cells/ml) to cowpea was only slightly less than its response for chickpea. Groundnut stimulated IC 59 to a lesser extent (7.6449 log cells/ml) while soybean showed the least stimulation (7.5873 log cells/ml). Among the non-legumes, maize had the highest count of IC 59 (7.5928 log cells/ml) followed by ragi (7.5873 log cells/ml) and cotton (7.5844 log cells/ml). Sunflower recorded the least increase in the population density of IC 59.

P 132 the cowpea specific rhizobial strain showed highest population increase in the cowpea root exudates (7.6506 log cells/ml) while the lowest population was found in sunflower (7.5234 log cells/ml). P 132 showed elevated counts in other legumes other than its host cowpea. Groundnut root exudate had only slightly lesser population densities (7.6418 log cells/ml) of P 132 than cowpea. The counts of P 132 was about the same for the three chickpea isolates and the Annageri cultivar (7.5873, 7.5835, 7.5711 and 7.5682 log cells/ml, respectively) though it was slightly less than

groundnut. Soybean had the least increase (7.5612 log cells/ml) among the legumes for P 132. Stimulation of P 132 to multiply was less in the non-legume root exudates than in the legume root exudates. Maize (7.548 log cells/ml) and ragi (7.5367 log cells/ml) maintained higher counts of P 132 than cotton (7.5267 log cells/ml) and sunflower (7.5234 log cells/ml).

The highest population of TAL 1000 rhizobial strain nodulating groundnut was found in the root exudates of groundnut (7.6115 log cells/ml) and the least population was found in the root exudates of sunflower (7.5325 log cells/ml). Cowpea root exudate was observed to have the second highest population (7.6056 log cells/ml) of TAL 1000 followed by the chickpea varieties. Soybean root exudates exhibited relatively lesser increase in the number of TAL 1000 (7.5740 log cells/ml) cells among the legumes. TAL 1000 counts were considerably lower in non-legumes than the legumes. A comparison showed that TAL 1000 cells multiplied more in the graminaceous root exudates of maize (7.5711 log cells/ml) and ragi (7.5607 log cells/ml) than in the root exudates of dicots, cotton (7.5420 log cells/ml) and sunflower (7.5325 log cells/ml).

Numbers of TAL 102 were highest in the root exudates of soybean (7.6056 log cells/ml) while the least population

density of TAL 102 was observed in the root exudates of sunflower (7.5604 log cells/ml). Relatively lower population counts were found in the other legumes cowpea (7.5855 log cells/ml) chickpea varieties (7.5807, 7.5749, 7.5667 and 7.5652 log cells/ml) and groundnut when compared to soybean. TAL 102 was comparatively less responsive in the root exudates of non-legumes with the population counts being (7.5652, 7.5632 and 7.5642 log cells/ml) for maize, cotton and ragi, respectively.

A crop-wise comparison showed that the root exudates of chickpea varieties were found to preferentially stimulate the multiplication of the IC 59 strain while there was no noticeable difference in the increased populations of other strains. TAL 102 was found to be relatively less stimulated in the root exudates of cowpea (7.5855 log cells/ml) when compared to IC 59 (7.6499 log cells/ml), TAL 1000 (7.6056 log cells/ml) and P 132 (7.6506 log cells/ml). IC 59 (7.6401 log cells/ml) and TAL 102 (7.6056 log cells/ml) multiplied better in the soybean root exudates when compared to P 132 (7.5612 log cells/ml) and TAL 1000 (7.5740 log cells/ml). TAL 1000 (7.6115 log cells/ml) a groundnut nodulating strain was found to be less stimulated in the groundnut root exudates when compared to the strains IC 59 and P 132 (7.6449 and 7.6418 log cells/ml, respectively). TAL 102 was least stimulated in the root exudate (7.591 log

cells/ml) of groundnut. Maize and ragi both stimulated IC 59 in their root exudates (7.5928 and 7.5873 log cells/ml, respectively) while P 132 (7.5481 and 7.5367 log cells/ml, respectively) was least stimulated in their rhizospheres. Sunflower root exudate stimulated IC 59 the highest (7.5835 log cells/ml) followed by TAL 102 (7.5604 log cells/ml) with P 132 being stimulated the least. A similar situation was found in cotton wherein P 132 and TAL 1000 were least stimulated (7.5267 and 7.5420 log cells/ml, respectively) while IC 59 was stimulated the greatest (7.5844 log cells/ml) (Fig. 1 and 2).

4.2 CHEMOTAXIS OF RHIZOBIA TOWARDS SEED LEACHEATES

Preferential stimulation of certain rhizobial strains by certain legume and non-legume root exudates led to the testing of chemotaxis of the rhizobial strains towards seed leacheates, germinating seed and root exudates.

Relative responses of Rhizobium strains of IC 59, P 132, TAL 1000 and TAL 102 towards seed leacheates of legumes and non-legumes was found to vary from crop to crop depending upon the rhizobial strain used. Rhizobial strains in general, showed greater relative response towards legumes than non-legumes (Table 6 and 7).

IC 59 Rhizobium strain showed greatest chemotaxis towards seed leacheates of chickpea (RR=20.91) and cowpea

Table 6: Chemotactic response (relative response*) of Rhizobium strains towards seed leacheates of legumes

Legumes	Rhizobial strains/Host legumes			
	IC 59/ Chickpea	P 132/ Cowpea	TAL 1000/ Groundnut	TAL 102/ Soybean
Chickpea	20.91	20.70	19.35	18.76
Cowpea	20.86	20.80	19.55	18.69
Soybean	19.78	19.70	19.10	18.84
Groundnut	19.83	19.48	19.99	18.55
Control	1.55	1.25	1.30	1.15

Table 7: Chemotactic response (relative response*) of Rhizobium strains towards seed leacheates of non-legumes

Non-legumes	Rhizobial strains/Host legumes			
	IC 59/ Chickpea	P 132/ Cowpea	TAL 1000/ Groundnut	TAL 102/ Soybean
Maize	19.25	17.40	17.18	17.25
Ragi	16.51	17.00	16.25	17.46
Sunflower	10.34	9.40	10.38	9.35
Cotton	10.41	13.33	13.27	14.35
Control	1.55	1.25	1.30	1.15

* Number of colony forming units per capillary containing exudates per number of colony forming units in the capillaries with control

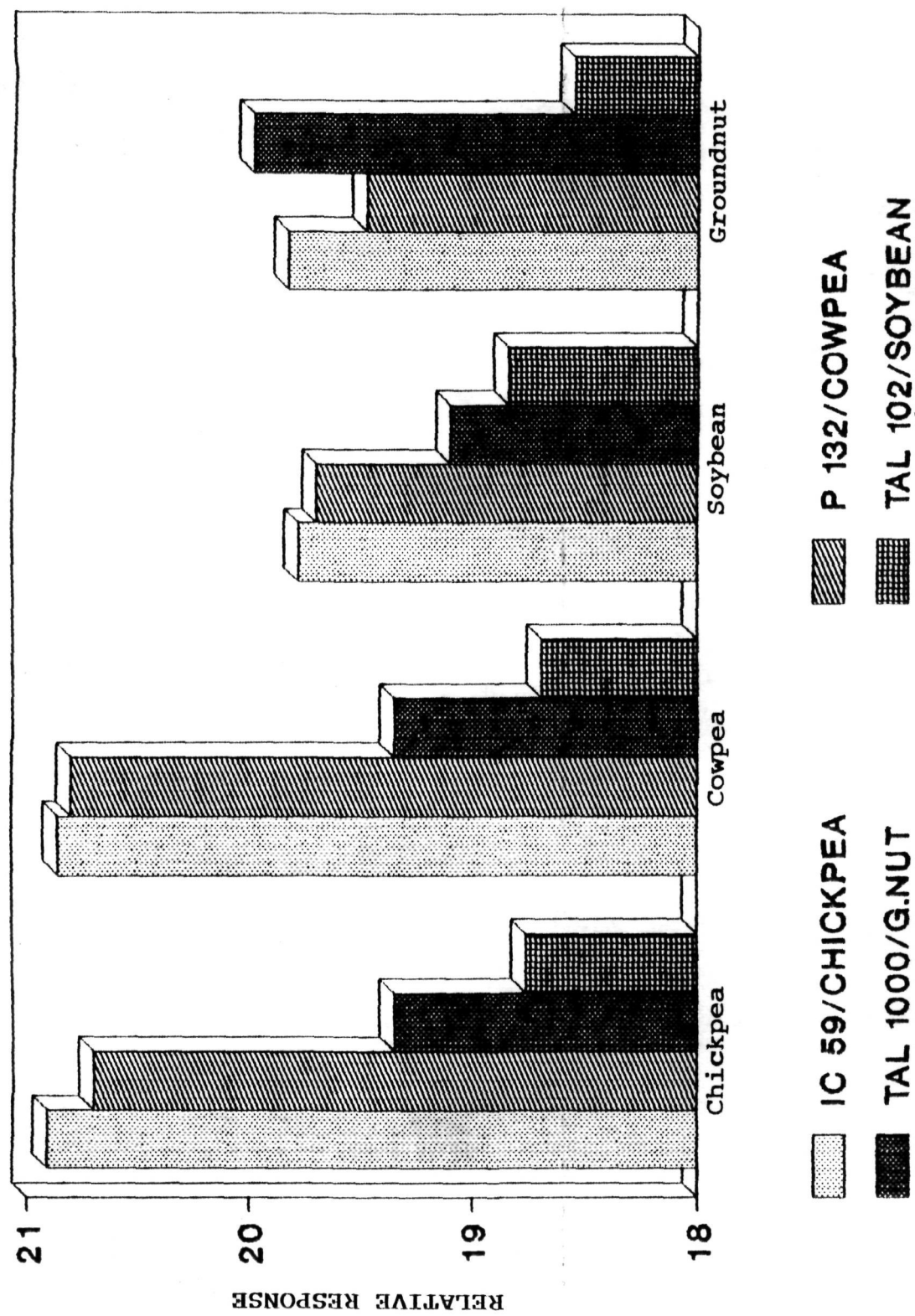


Fig. 3: CHEMOTACTIC (RELATIVE RESPONSE) RESPONSE OF RHIZOBIUM STRAINS TOWARDS SEED LEACHEATES OF LEGUMES

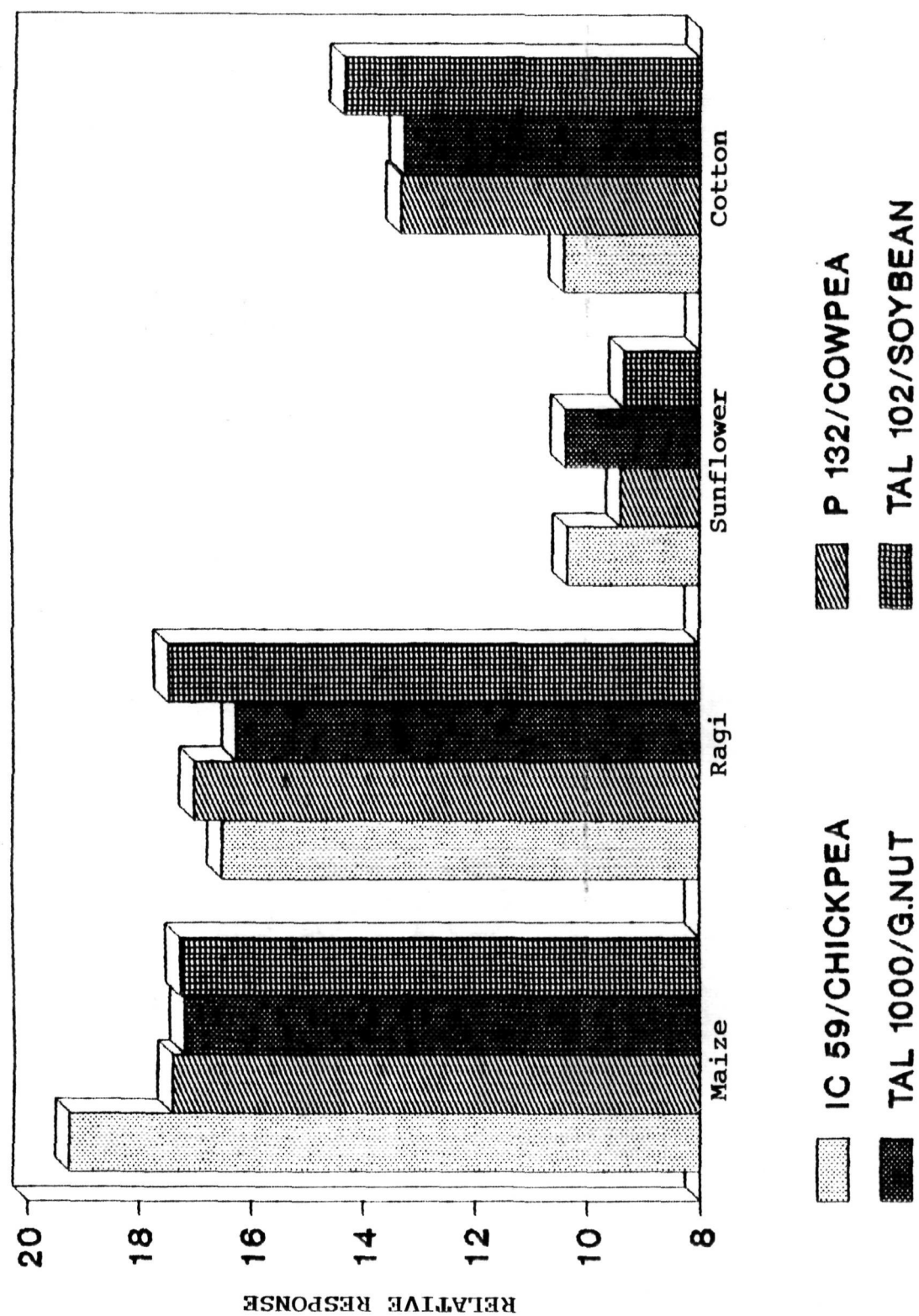


Fig. 4: CHEMOTACTIC RESPONSE (RELATIVE RESPONSE) OF *RHIZOBIUM* STRAINS TOWARDS SEED LEACHATES OF NON-LEGUMES

(RR=20.86) while the lowest chemotaxis was towards seed leacheate of sunflower (RR=10.34). Soybean (RR=19.78) and groundnut (RR=19.83) seed leacheates evoked a slightly lesser chemotactic response than chickpea. Non-legume seed leacheate evoked considerably low but positive chemotaxis. Relative responses of IC 59 was the highest for maize (RR=19.247) followed by ragi (RR=16.505), cotton (RR=10.408) and sunflower. Seed leacheates of chickpea (RR=20.7), cowpea (RR=20.8) and groundnut (RR=10.04) were found to evoke high comparable chemotaxis responses from P 132 while the least chemotactic response of P 132 was observed towards sunflower seed leacheate (RR=9.4). Of the legumes studied for chemotactic response soybean seed leacheate evoked the least (RR=19.7) response from P 132. Among the non-legumes, maize (RR=17.4) and ragi ((RR=17.0) elicited relative responses greater than cotton (RR=13.332) and sunflower (RR=9.4) seed leacheates.

Maximum attraction of TAL 1000 was found to be towards the legume seed leacheate of cowpea (RR=19.55) and groundnut (RR=19.99) while chickpea (RR=19.35) and soybean (RR=19.10) seed leacheates were also found to be attractants evoking a slightly lesser chemotactic response. Non-legume seed leacheates elicited comparatively lesser chemotactic responses from TAL 1000 with maize (RR=17.17) and ragi (RR=16.25) seed leacheates proving to be better attractants

than seed leacheates of cotton (RR=13.26) and sunflower (RR=10.384) which evoked the least relative responses from TAL 1000.

Sunflower seed leacheate evoked the least chemotactic response (RR=9.34) from TAL 102 while soybean (RR=18.84) evoked the greatest response. Responses of TAL 102 to chickpea, cowpea and groundnut seed leacheates (RR=18.76, RR=18.69 and RR=18.55, respectively) varied slightly and were comparable among themselves while maize and ragi seed leacheates evoked almost similar (17.24 and 17.46, respectively) relative responses, with cotton seed exudate being a weaker attractant with a relative response of 14.347.

Chickpea, cowpea and soybean (RR=20.91, RR=20.86 and RR=21.98, respectively) were found to evoke a high preferential chemotaxis from IC 59 and P 132 strains. Soybean, specific TAL 102 strain showed lesser (RR=18.84) chemotactic response to its host soybean. Groundnut seed leacheate elicited greater (RR=19.99) chemotactic response from groundnut specific strain TAL 1000 as also from IC 59 (RR=19.83). IC 59 showed greater chemotactic response towards maize seed leacheate (RR=19.24) compared to other rhizobial strains which exhibited almost similar chemotactic responses towards maize seed leacheate. Ragi seed leacheate evoked a lesser chemotactic response from IC 59 (RR=16.50) while the greatest response was evoked from TAL 102

(RR=17.46) followed by P 132 (RR=17.0). Rhizobial strains IC 59 (RR=10.34) and TAL 1000 (RR=10.38) showed similar relative responses towards the seed leacheate of sunflower while P 132 (RR=9.4) and TAL 102 (RR=9.35) exhibited a lesser response. A higher relative response (RR=14.347) was shown by TAL 102 towards the seed leacheate of cotton while IC 59, which had showed greater relative responses to the seed leacheates of other crops, was found to have the least chemotactic responses with a relative response of 10.40 (Fig. 3 and 4).

4.3 EFFECT OF GERMINATING SEEDS (SPERMOSPHERE) ON RHIZOBIAL STRAINS

Chemotaxis of Rhizobium strains towards seed leacheates of legumes and non-legumes were observed. So the effect of germinating seeds on the rhizobial strains were studied by Agar plate testing of inhibition (Table 8).

Irrespective of the crop both legumes and non-legumes showed inhibition of rhizobia (Plate 2 to 9). The degree of inhibition varied according to the seeds and the rhizobia. The inhibition of TAL 102 by cotton germinating seeds (33 mm) was highest while the spermosphere of groundnut inhibited P 132 the least (12 mm). IC 59 was inhibited the most by soybean (30 mm) and the least by maize (1.5 mm). Among the legumes the diameter of the zone of inhibition of the strain

Table 8: Inhibition of rhizobia by diffusates from
germinating legume and non-legume seeds

Legumes/ Non-legumes	Diameter of inhibition (mm)			

	Rhizobial strains/Host legumes			
	IC 59/ Chickpea	P 132/ Cowpea	TAL 1000/ Groundnut	TAL 102/ Soybean
Chickpea	20.0	23.5	22.0	25.0
Cowpea	22.5	18.0	19.0	20.0
Soybean	30.0	25.5	23.5	21.0
Groundnut	20.0	12.0	13.5	20.5
Maize	15.0	20.3	23.3	18.0
Ragi	22.5	16.0	20.3	13.5
Sunflower	27.0	32.0	27.0	29.0
Cotton	25.0	29.0	30.3	33.0

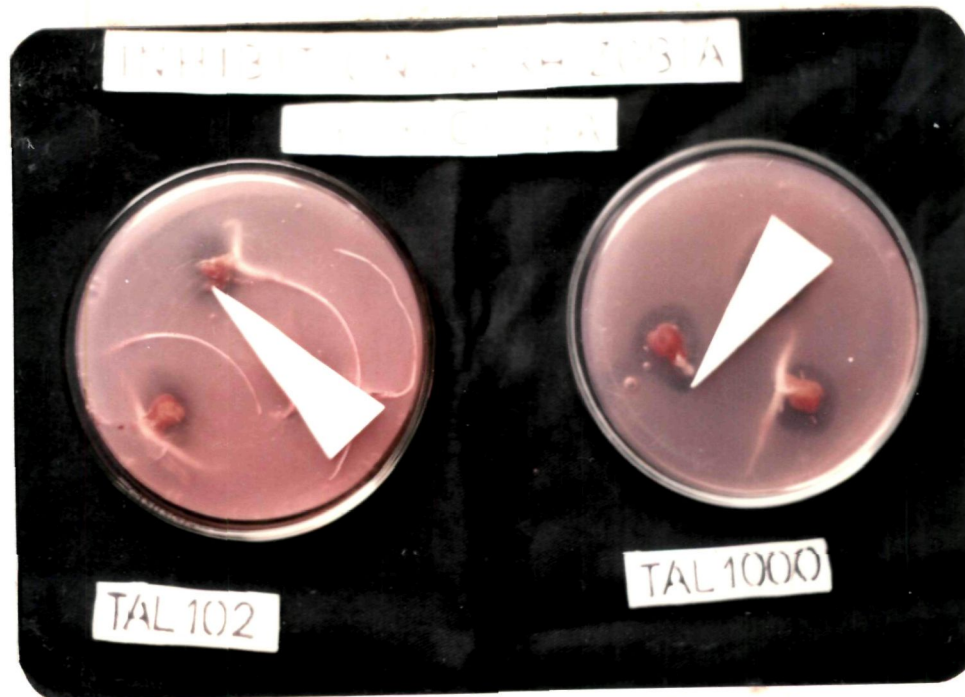


Plate 2: Inhibition of rhizobial strains by chickpea



Plate 3: Inhibition of rhizobial strains by cowpea



Plate 4: Inhibition of rhizobial strains by soybean



Plate 5: Inhibition of rhizobial strains by groundnut



Plate 6: Inhibition of rhizobial strains by maize

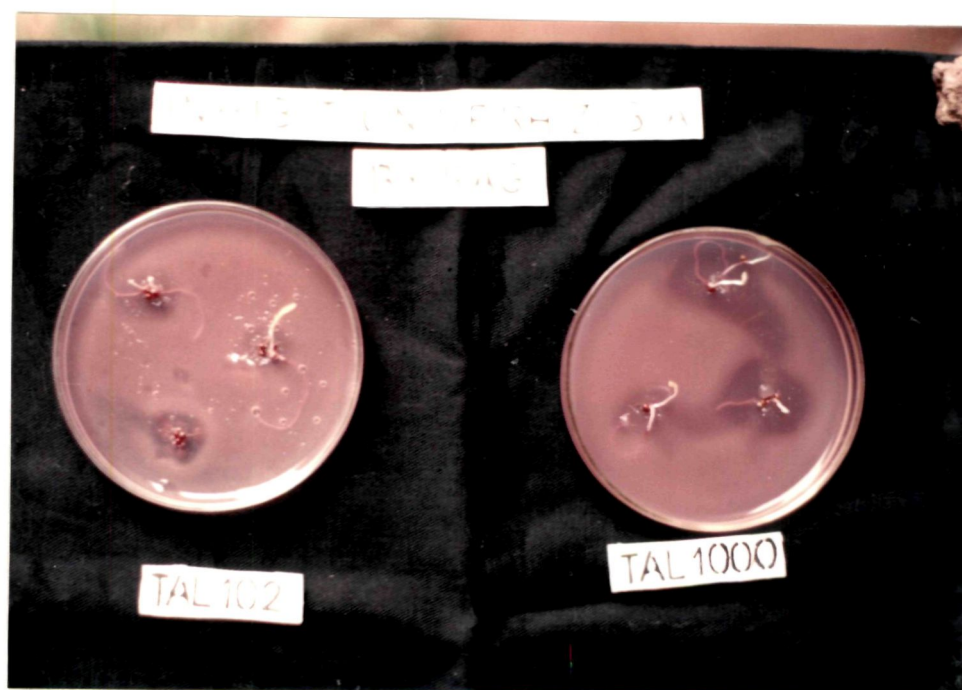


Plate 7: Inhibition of rhizobial strains by ragi



Plate 8: Inhibition of rhizobial strains by sunflower



Plate 9: Inhibition of rhizobial strains by cotton

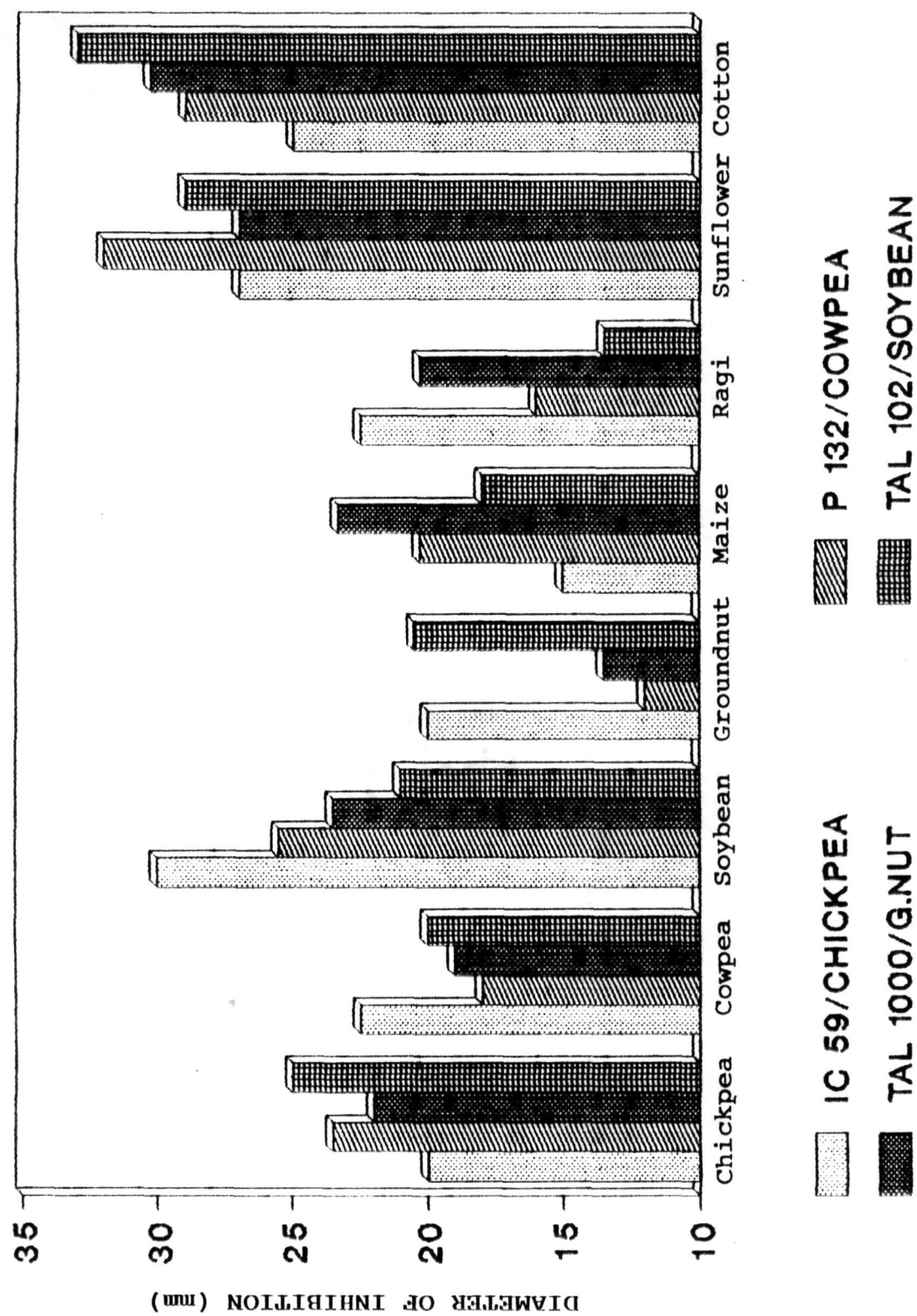


Fig. 5: INHIBITION OF RHIZOBIA BY DIFFUSATES FROM GERMINATING LEGUME AND NON-LEGUME SEEDS

IC 59 were thus: the minimum being 20.0 mm for chickpea and groundnut, 22.5 mm for cowpea, and the maximum 30 mm for soybean. Inhibition of IC 59 by non-legumes produced zones varying from a minimum of 15 mm by maize and a maximum of 27 mm by sunflower. Inhibition of P 132 was the highest by sunflower (32.0 mm) and the lowest by groundnut (12 mm). Exudates from germinating chickpea and soybean markedly inhibited the growth of P 132 with the mean inhibition being 23.5 mm and 25.5 mm, respectively while groundnut and cowpea showed comparatively lower inhibition (12.0 mm and 18.0 mm, respectively). The zone of rhizobial growth (P 132) inhibition was the highest for sunflower (32.0 mm) followed by cotton (29 mm) and ragi (16 mm). TAL 1000 was inhibited the most by cotton (30.3 mm) and the least by groundnut (3.5 mm). Among the legumes, radii of the zones of inhibition of TAL 1000 were the highest for soybean (23.5 mm) and the lowest for groundnut (13.5 mm). TAL 1000 was found to be more sensitive to non-legume germinating seed exudates with the least toxic being exudates from the germinating ragi seed (23.3 mm) while the most toxic was cotton (30.3 mm). Cotton inhibited TAL 102 the most (33.0 mm) while exudates of germinating maize inhibited the least (18.0 mm). Germinating cowpea seed exudates, among the legumes were less inhibitory (20.0 mm) to the rhizobial strain TAL 102 than were the exudates of groundnut, soybean

and chickpea, which had average zones of inhibition as 2.5 mm, 21.0 mm and 25.0 mm, respectively. Non-legumes, sunflower and cotton strongly inhibited TAL 102 with inhibition zones of 29.0 mm and 33.0 mm, respectively while the inhibition by ragi and maize were slightly lower (13.5 and 18.0 mm, respectively).

The spermosphere of chickpea (23.5 mm) inhibited P 132 to the greatest extent while IC 59 was inhibited the least. P 132 a cowpea specific strain was inhibited the least (18.0 mm) while IC 59 was inhibited the maximum (22.5 mm) by cowpea seeds. Soybean inhibited IC 59 to the maximum extent (30.0 mm) followed by P 132 (25.5 mm) and TAL 1000 (23.5 mm). Cowpea specific P 132 was found to be the least inhibited (12.0 mm) by groundnut germinating seeds followed by TAL 1000, and the maximum inhibition was observed for IC 59 (20.0 mm) and TAL 102 (20.5 mm). Inhibition of rhizobial strains by germinating seeds of maize was the highest for TAL 1000 (23.3 mm) and the least for IC 59 (15.0 mm). Germinating seeds of ragi inhibited IC 59 the most (22.5 mm) and TAL 102 the least (13.5 mm). In general, the spermosphere of cotton inhibited all the rhizobial strains to higher extent. The highest inhibition was towards TAL 102 (33.0 mm) and the least inhibited was IC 59 (25.0 mm). Similarly, sunflower spermosphere also showed higher inhibition towards all rhizobial strains with the highest inhibi-

tion (32.0 mm) of P 132 followed by TAL 102 (29.0 mm) while IC 59 strain and TAL 1000 showed inhibition zones of 27.0 mm (Fig. 5).

4.4 CHEMOTAXIS OF RHIZOBIAL STRAINS TOWARDS ROOT EXUDATES

Chemotaxis of rhizobial strains towards seed leacheates were observed but the germinating seeds were found to inhibit rhizobia. So a study was conducted to test the chemotaxis of the rhizobial strains IC 59, P 132, TAL 1000 and TAL 102 towards the root exudates of legumes and non-legumes.

In general, the relative response of rhizobial strains towards root exudates (Av. 48) was greater when compared to the seed exudates. The relative responses towards the root exudates ranged from a maximum of 55.48 observed for IC 59 for chickpea root exudates to a minimum of 38.86 by TAL 102 towards sunflower root exudate. All the exudates evoked positive responses though the non-legume root exudates always produced a slightly lower response than the legume root exudates (Table 9 and 10).

Amongst the legumes, chickpea (RR=55.48) and cowpea root exudates (RR=55.64) were stronger chemoattractants for IC 59 than soybean (RR=54.42) and groundnut (RR=54.29) root exudates. Of the non-legumes tested maize root exudate evoked a chemotactic response from IC 59 (RR=54.36) almost equal to that of groundnut. Ragi (RR=53.91) and cotton

Table 9: Chemotactic response (relative response*) of Rhizobium strains towards root exudates of legumes

Legumes	Rhizobial strains/Host legumes			
	IC 59/ Chickpea	P 132/ Cowpea	TAL 1000/ Groundnut	TAL 102/ Soybean
Chickpea	55.48	52.43	53.00	47.50
Cowpea	55.64	53.61	53.53	48.29
Soybean	54.42	52.36	53.20	48.48
Groundnut	54.29	53.47	53.33	47.65
Control	1.30	1.20	1.25	1.10

Table 10: Chemotactic response (relative response*) of Rhizobium strains towards root exudates of non-legumes

Non-legumes	Rhizobial strains/Host legumes			
	IC 59/ Chickpea	P 132/ Cowpea	TAL 1000/ Groundnut	TAL 102/ Soybean
Maize	54.36	52.43	53.26	47.12
Ragi	53.91	52.29	53.13	45.98
Sunflower	43.20	40.29	42.27	38.86
Cotton	52.37	52.22	52.40	45.98
Control	1.30	1.20	1.25	1.10

* Number of colony forming units per capillary containing exudates per number of colony forming units in the capillaries with control

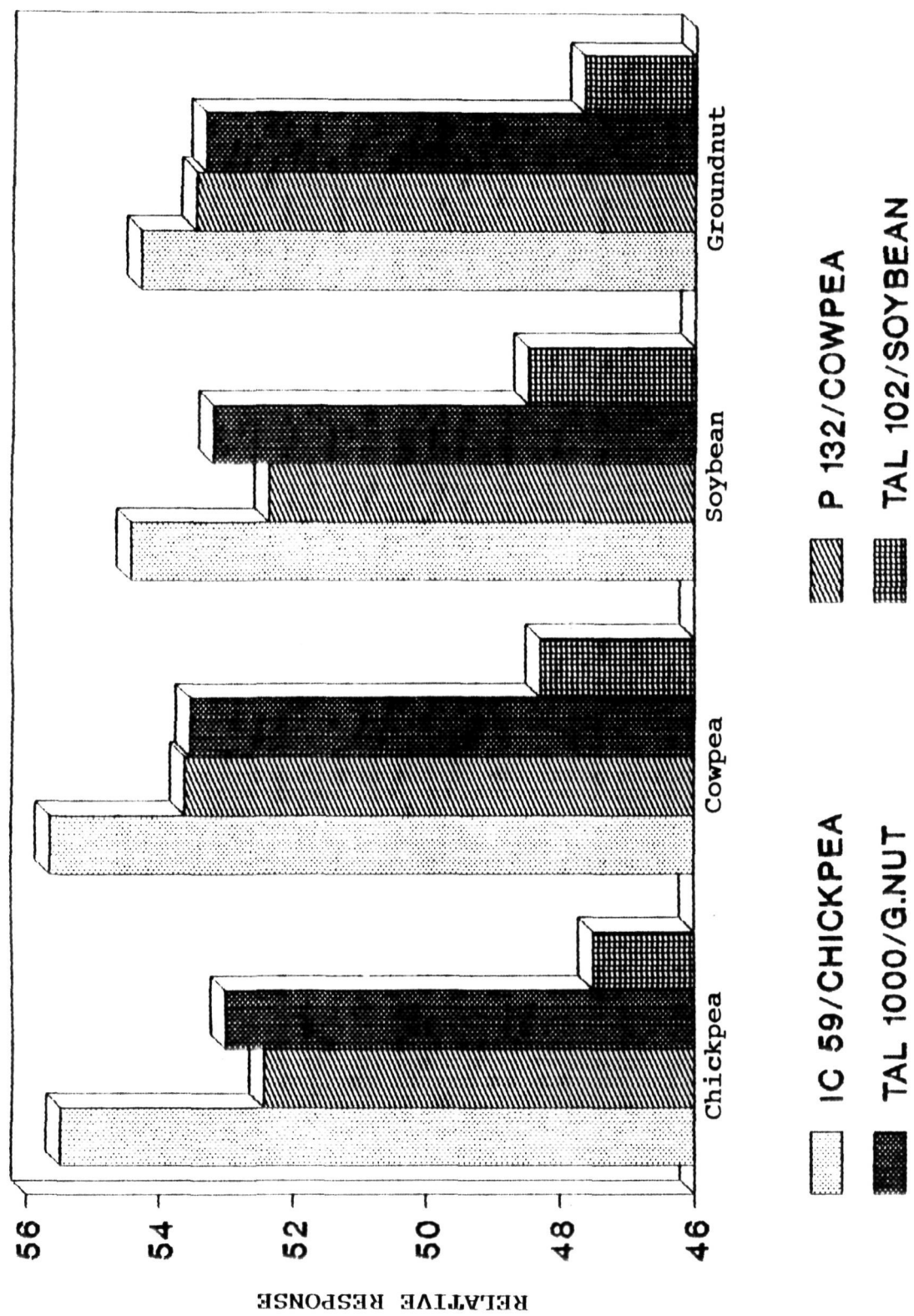


Fig. 6: CHEMOTACTIC RESPONSE (RELATIVE RESPONSE) OF RHIZOBIUM STRAINS TOWARDS ROOT EXUDATES OF LEGUMES

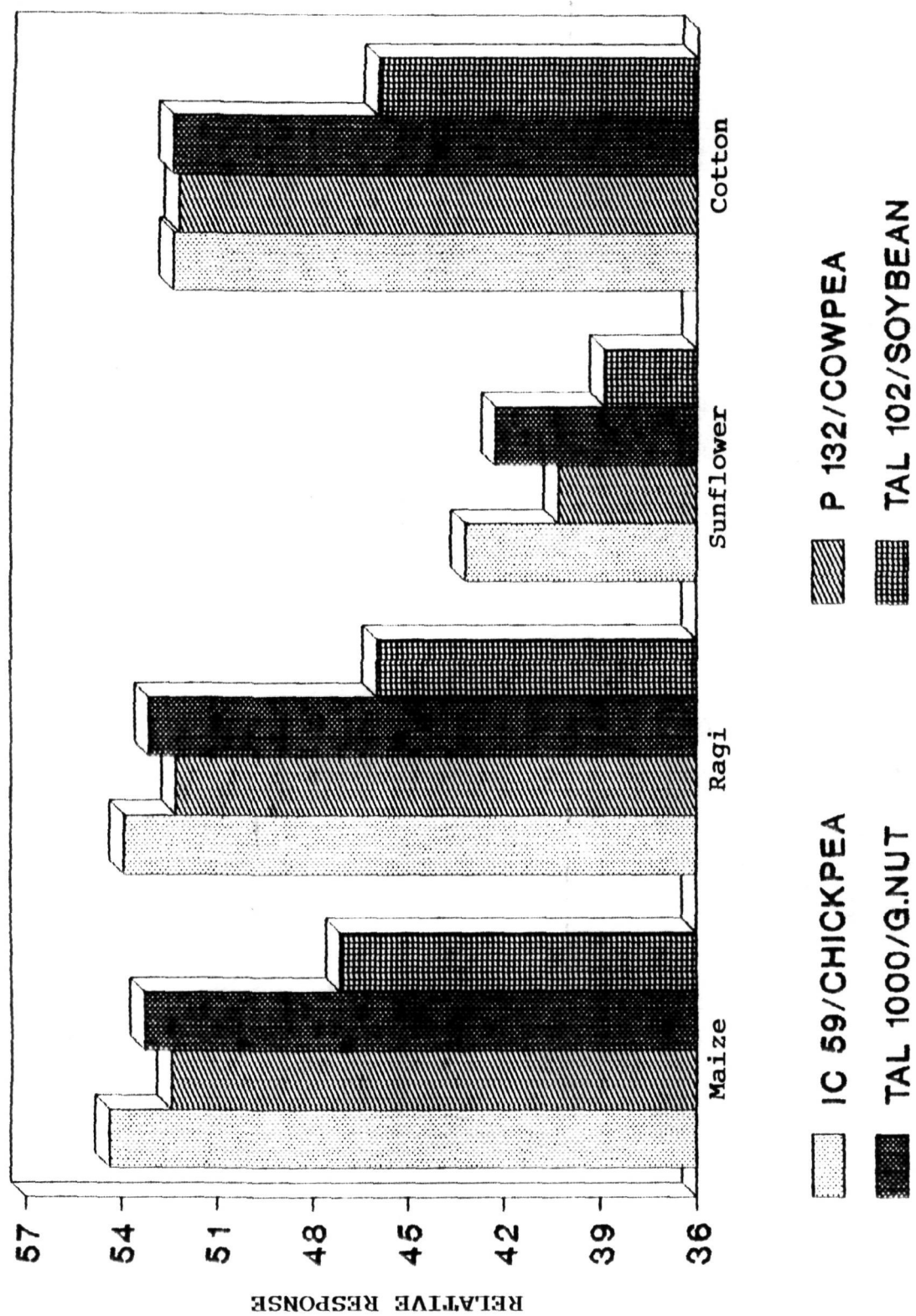


Fig. 7: CHEMOTACTIC RESPONSE (RELATIVE RESPONSE) OF *RHIZOBIUM* STRAINS TOWARDS ROOT EXUDATES OF NON-LEGUMES

(RR=52.371) evoked a better response from IC 59 than sunflower (RR=43.20).

The chemotactic behavior of P 132 to the root exudates was as follows: Cowpea (RR=53.61) and groundnut root exudates (RR=53.472) were found to be the best attractants among legumes followed closely by chickpea (RR=52.43) and soybean (RR=52.36) root exudates. Chemotactic responses of P 132 towards the non-legumes of maize (RR=52.43), ragi (RR=52.29) and cotton (RR=52.22) were found to be almost equal to its response to soybean root exudates. Sunflower root exudates (RR=40.29) elicited a comparatively lower chemotactic response than the other non-legumes.

All the legume and non-legume root exudates except sunflower were found to be strong chemoattractants for TAL 1000. Chickpea (RR=53.40), cowpea (RR=53.53), soybean (RR=53.2) and groundnut (RR=53.472) elicited almost similar responses from TAL 1000 while the non-legumes elicited a slightly lower relative response of 53.22 for maize, 53.12 for ragi and 52.4 for cotton. TAL 1000 demonstrated a lower but still positive chemotaxis (RR=42.26) to sunflower root exudate.

Relative responses of TAL 102 was highest for soybean root exudate (RR=48.48) closely followed by cowpea (RR=48.29), groundnut (RR=47.65) and chickpea (RR=47.5).

Among the non-legumes chemotaxis of TAL 102 towards maize root exudates (RR=47.12) was slightly lesser than the legumes. Ragi and cotton root exudates (RR=45.98) elicited the same relative response from TAL 102. Sunflower root exudate was found to have the lowest (RR=38.86) chemoattraction among non-legumes for TAL 102.

IC 59 the chickpea specific Rhizobium strain was found to be more chemotactic towards the root exudates of its host (RR=55.48) while TAL 102 responded the least (RR=47.5) towards the root exudates of chickpea. IC 59 showed greater response (RR=55.65) towards cowpea root exudates while P 132 (RR=53.6) and TAL 1000 (RR=53.53) showed an almost similar response towards cowpea root exudates. Chemotaxis of IC 59 (RR=54.42) towards the root exudate of soybean was found to be the highest followed by TAL 1000 (53.2) and P 132 (RR=52.36). While TAL 102 a soybean specific strain exhibited the least (RR=48.48) chemotactic response. The relative response of P 132 and TAL 1000 (RR=53.47 and RR=54.29, respectively) towards root exudates of groundnut was almost equal while IC 59 showed the highest chemotaxis (RR=54.29). IC 59 showed preferential chemotaxis towards maize root exudates (RR=54.35) while TAL 102 was attracted the least towards maize root exudates (RR=47.12). Root exudates of ragi preferentially evoked greater chemotactic response from IC 59 (RR=53.91) and TAL 1000 (RR=53.33) and a minimum

response of 45.98 from TAL 102. Sunflower root exudates showed higher chemotaxis for IC 59 (RR=53.20) followed by TAL 1000 (RR=42.26), P 132 (RR=40.27) and TAL 102 (RR=28.86). Rhizobial strains IC 59 (RR=52.37), P 132 (RR=52.22) and TAL 1000 (RR=52.4) showed almost similar relative responses to the root exudates of cotton. TAL 102 (RR=45.98) responded the least towards root exudates of cotton (Fig. 6 and 7).

4.5 AMINO ACIDS PRESENT IN THE SEED LEACHEATES AND ROOT EXUDATES OF LEGUMES AND NON-LEGUMES

Since amino acids are one of the major constituents of the seed leacheates and root exudates, a study was done to identify the amino acids present in the seed leacheates and the root exudates of legumes and non-legumes. All the leacheates/exudates of legumes and non-legumes contained aspartic acid, glutamic acid and alanine. Glycine and isoleucine were the other more frequently occurring amino acids as they were found in the seed leacheates of cowpea, soybean, maize and ragi. Glycine was also found in cotton seed leacheates while isoleucine was found in chickpea seed leacheates. In addition, phenyl alanine and lysine were found in chickpea seed leacheates, serine and lysine in cowpea seed leacheates, leucine and lysine in groundnut leacheates. Phenyl alanine and thiamine were not found in

Table 11: Amino acids present in the seed leacheates of
legumes and non-legumes

Amino acids	Plant species							
	Chick- pea	Cow- pea	Soy- bean	G. nut	Maize	Ragi	Sun- flower	Cotton
Ala	+	+	+	+	+	+	+	+
Asp	+	+	+	+	+	+	+	+
Glu	+	+	+	+	+	+	+	+
Gly		+	+		+	+		
Ile	+	+	+		+	+	+	
Leu				+				+
Lys	+	+		+				
Phe	+		+	+	+		+	
Ser		+					+	+
Thr					+			
Val			+				+	

+ Presence in seed leacheates

cotton leacheate. Valine in ragi, phenyl alanine, serine and valine in sunflower, serine and leucine in cotton were the other amino acid constituents in non-legume seed leacheates (Table 11).

The most frequently occurring amino acids in the root exudates were aspartic acid, glutamic acid and alanine while isoleucine and phenyl alanine was detected in all the exudates except ragi and cowpea, respectively. Glycine was absent in the root exudates of chickpea, groundnut and sunflower while threonine was found only in the root exudates of chickpea and sunflower. In addition, serine was found in cowpea, ragi, cotton and sunflower root exudates and lysine in chickpea, cowpea, soybean and groundnut root exudates. Valine was found in all the root exudates except cowpea and cotton while arginine was present only in the root exudate of cowpea, ragi and cotton. Histidine and proline were found only in the root exudate of groundnut and maize, respectively (Table 12).

A comparison between the seed leacheates and the root exudates showed that threonine and valine were found only in the root exudates of chickpea but not in the seed leacheates. Arginine and lysine were found in the root exudates of cowpea and soybean, respectively but were not identified in their respective seed leacheates. Histidine and valine present in the root exudates of groundnut were

Table 12: Amino acids present in the root exudates of
legumes and non-legumes

Amino acids	Plant species							
	Chick- pea	Cow- pea	Soy- bean	G. nut	Maize	Ragi	Sun- flower	Cotton
Ala	+	+	+	+	+	+	+	+
Arg		+				+	+	
Asp	+	+	+	+	+	+	+	+
Glu	+	+	+	+	+	+	+	+
Gly		+	+		+	+	+	
His				+				
Ile	+	+	+		+		+	+
Lys	+	+	+	+				
Phe	+		+	+	+	+	+	+
Pro					+			
Ser		+				+	+	+
Thr	+							+
Val	+		+	+	+	+		+

+ Presence in root exudates

not present in the seed leacheates. Threonine found in maize seed leacheate was not found in the root exudate while the root exudate constituents valine and proline were not found in the seed leacheate. The root exudates of ragi contained the amino acids serine and arginine which were not identified in the seed leacheates. Phenyl alanine and arginine identified in the root exudates of cotton were absent in the seed leacheate while there was no difference in the composition of the sunflower seed leacheate and root exudates except for the presence of threonine in the sunflower root exudate.

DISCUSSION

V. DISCUSSION

Many attempts have been made to explain the specificity of the legume-Rhizobium symbiosis. Legume nodulation is said to be substantially controlled by the host. The early events of infection and nodulation of legumes by rhizobia are greatly influenced by the quantity and quality of exudates secreted by germinating seeds and young seedlings. Since visible root nodules are found within 10-15 days of germination of inoculated seed under optimum conditions it is important to examine the root exudates of germinating seed and young seedlings for their effect on the early events of rhizobial infection. The present investigation was conducted to determine whether the plant selects its homologous rhizobia by means of an interaction through the root exudates in the rhizosphere at the very early stages.

5.1 EFFECT OF LEGUME ROOT EXUDATES ON THE GROWTH OF RHIZOBIA

It is known that host legume root does elicit a rhizosphere effect on its specific rhizobia. However, rhizosphere effects of heterologous legumes and non-legumes on rhizobia is not much studied. In this experiment, growth of specific Rhizobium in the root exudates of its host relative to that of another Rhizobium representative of different

inoculation group was studied. The growth responses were similarly studied in non-host legume and non-legume root exudates.

The results of the study show that all the rhizobial strains IC 59, P 132, TAL 1000 and TAL 102 multiplied from 3.0 log number of cells per ml to an average of 7.6493 log number of cells per ml in the host root exudates. In the non-host legumes the average growth of the rhizobial strains were 7.5894 cells per ml, which is comparatively less than its multiplication in the root exudates of host legume. In the non-legume root exudates the increase was an average of 7.5577 log cells per ml. Among the non-legumes there was more growth in the root exudate of monocots than in the dicots. The growth of the rhizobial strains in the root exudates suggest that both legume and non-legume root exudates contain substances suitable for the multiplication of rhizobia. The multiplication of rhizobia, though slightly preferential in the host root exudate, is essentially the same in the presence of the host and non-host legumes. This indicates that there is no selective effect of the host legume root exudates on the infective symbiont which supports the laboratory observations made by Alexander and Peters (1966) using a system of seven rhizobia and their respective host legumes.

Although the concept of specific stimulation has received support in the past (Nutman, 1969), most available data (under field conditions) indicate that the rhizosphere stimulation might be non-specific (Tazimura and Watanabe, 1962). Similarly, Reyes and Schmidt (1979, 1981) and Robert and Schmidt (1983) studied specific stimulation of R. japonicum and R. phaseoli in soybean and pea root rhizospheres, respectively and found that, although rhizobia generally grew well in the host rhizospheres, the stimulation was not specific for the particular legume-Rhizobium system tested.

There was very little difference in the growth of rhizobia (both homologous and non-homologous) in the root exudates of the high nodulating, medium nodulating and the low nodulating isolates of chickpea. A similar observation was also made in soybean isolates by Clarke (1957) and Reyes and Schmidt (1979). On the other hand Mathews et al. (1993) reported that supernodulating soybean supported substantially large population of R. japonicum in their seedling rhizospheres.

Thus, extensive colonization of the microsymbiont in the host rhizosphere may not be a factor leading to the specificity of the symbiosis. The ability of the rhizobia to grow in the root exudates of non-host plants may also serve to maintain indigenous population of the rhizobia in

the soil, though at slightly lower densities than in the absence of the host plant.

This study indicates that rhizobia can survive and multiply in soils as long as any crop or even weeds are grown. This is contrary to the hypothesis that cultivation of legumes increases the population of rhizobia. Logically rhizobia as a saprophyte should multiply like any other soil bacteria as long as nutrients become available through plant root exudates, the question being saprophytic competence. In the present study rhizobial growth is studied under axenic conditions. Thus, this will not give an idea of their survival and growth in natural situations. It is possible that though rhizobia can utilize non-legume root exudates for growth they may not stand competition from other soil bacteria resulting in their slow elimination in the absence of legumes specially homologous legumes. When legumes are cultivated the rhizobia are safe inside the host root, in nodules and on nodule senescence their population increases in the soil. It would be interesting if any non-legume crop or even a weed is identified which can specifically stimulate the growth of rhizobia or conversely specifically inhibit soil bacteria other than rhizobia, such a crop will help in increased exploitation of symbiotic nitrogen fixation for crop production.

5.2 CHEMOTAXIS OF RHIZOBIA TOWARDS SEED LEACHEATES AND ROOT EXUDATES

The previous experiment indicates that the rhizobia survive and multiply in not only in the host legume but also in the non-host legume and the non-legume root exudates. Specific chemotaxis between the host plant root exudates and the rhizobia is thought to be involved in the rhizobial colonization of the host rhizosphere by attracting and allowing only the homologous rhizobia to arrive at the host root zone. Chemotaxis towards leacheates/exudates has been suggested to be the first step in the colonization of roots during infection. The chemotactic ability of Rhizobium to seed leacheates and plant root exudates obtained from four legumes and non-legumes were tested by capillary tube method in this experiment.

The results showed positive chemotaxis of all the rhizobia towards all the seed leacheates and root exudates. Root exudates elicited a better chemotactic response (RR=49.89) from the rhizobia than the seed leacheates (RR=17.00). The legume seed leacheate (RR=19.68) and root exudates (RR=51.04) proved to be better attractants than non-legume seed leacheate (RR=14.19) and root exudates (RR=48.75). Among the non-legumes the dicot seed leacheates (RR=11.34) and root exudates (RR=45.948) were weaker attractants than the seed leacheates (RR=17.30) and root

exudates (RR=51.56) of ragi and maize. The chemotaxis indicates no specific attraction of rhizobia towards its specific host.

Such chemotactic responses of rhizobia towards legumes, non correlated with nodulation, and even towards non-legumes has been reported by several workers (Currier and Strobel, 1976; Gaworzewska and Carlile, 1982; Ahn and Lee, 1989). The results clearly support that legume and non-legume seed leacheate and root exudate contain substances that attract rhizobia and that there is non-specific migration of rhizobia towards the exudates of a wide array of legumes and non-legumes. Though non-specific, chemotaxis towards the seed leacheates and root exudates may play a role in the structuring of the microbial community around the developing root. However, chemotaxis is thought to provide a competitive advantage to the rhizobia while competing for nodulation sites on the host root.

The present study shows that the chemotaxis phenomenon has little role to play in the specificity of legume-Rhizobium symbiosis. Rhizobia are attracted towards most root exudates like other soil bacteria and the specificity mechanism starts beyond the attraction phase. However, attraction of rhizobia to the infection site is important in the infection process. Flavonoid compounds in the root

exudates are implicated in both attraction and specificity (recognition) of symbiosis. Flavonoids are also known for nod gene induction in rhizobia.

5.3 AMINO ACID COMPOSITION OF THE SEED LEACHEATES AND ROOT EXUDATES

Amino acids have been reported to be the dominant components in the seed leacheates and root exudates (Gitte et al., 1978; Rai and Strobel, 1966; Gaworzewska and Carlile, 1982). Amino acids were also suggested to be specific for a particular plant species or plant family (Vancura, 1980). Specific amino acids secreted by the host plant roots, for example homoserine secretion by pea roots is thought to selectively stimulate its homologous rhizobia (VanEgeraat, 1975). Thus, in this study the amino acid composition of the seed leacheates and root exudates of the various legumes and non-legumes were determined.

In the experiment it was found that there was no difference between the seed leacheates and root exudates of the crops. The chickpea seed leacheate and the root exudate contained aspartic acid, glutamic acid, alanine, isoleucine, phenyl alanine and lysine. Threonine and valine were found in the root exudates only. A similar amino acid profile for the root exudates of chickpea has been reported by Rai and Prasad in 1986. Cowpea seed leacheate and root exudate

contained aspartic acid, glutamic acid, alanine, glycine, isoleucine, serine and lysine while arginine was found only in the root exudates. Aspartic acid, glutamic acid, alanine, glycine, isoleucine, phenyl alanine and valine were detected in the seed leacheate and root exudate of soybean. An additional amino acid lysine was found in root exudates of soybean. These amino acids were also identified by Barbour et al. (1991) in the soybean seed leacheate and root exudate. Seed leacheates and root exudates of groundnut were found to contain aspartic acid, glutamic acid, alanine, phenyl alanine and lysine. Leucine was found only in the seed leacheate while valine and histidine were found in the root exudate only. Subrahmanyam et al. (1983) have reported the presence of these amino acids along with many other amino acids in the seed leacheates of groundnut.

Vancura (1967) reported the presence of many amino acids in the seed leacheate of maize, like aspartic acid, glutamic acid, alanine, glycine, isoleucine, phenyl alanine and threonine, which were also identified in the study as the components of both the seed leacheate and root exudate of maize. Ragi seed leacheate and root exudate were found to contain aspartic acid, glutamic acid, alanine and valine. The seed leacheates also had isoleucine while the root exudates had a few additional amino acids like phenyl alanine, serine and arginine. Sunflower seed leacheates and

root exudates had aspartic acid, glutamic acid, alanine, glycine, isoleucine, phenyl alanine and serine. The seed leacheates also contained valine while arginine was found in the root exudates only. Most of these amino acids were also identified in the sunflower root exudates by Frenzel (1957). Cotton seed leacheate and root exudate contained aspartic acid, glutamic acid, alanine and serine. The seed leacheates also contained leucine while isoleucine, phenyl alanine, threonine and valine were identified in the root exudates. Most of the amino acids identified in the root exudates have also been detected earlier by Sulochana (1962).

Thus, aspartic acid, glutamic acid and alanine were common to all the root exudates and seed leacheates. The other frequently occurring amino acids were isoleucine and phenyl alanine followed by glycine and valine. In this experiment it was found that lysine occurred only in the legume exudates but not in the non-legume exudates though it has been reported by other workers in oats (Katznelson et al., 1955), rice (Frank, 1954), wheat (Katznelson et al., 1955) and cotton (Sulochana, 1962). Such differences in the amino acid composition may occur due to the different varieties used and also due to the different methods of collection.

It was observed that there was very little difference in the seed and seedling exudates. The exudates of the different crops were also not qualitatively distinct as to the presence of various amino acids.

Thus, the ubiquitous presence of amino acids in both the legume and the non-legume exudates indicate that the presence of exudate amino acids is not an important criteria for the legume-Rhizobium specificity. The presence of the same amino acids in the non-legume exudates might be the reason for the growth and multiplication of rhizobia in the root exudates of non-legumes. In the past also the only example of an amino acid involved in legume-Rhizobium specificity is found with pea root exudate homoserine attracting and stimulating growth specifically of R. leguminosarum. It may thus be possible that flavonoids are more important than amino acids in recognition and nodulation phenomenon.

5.4 INHIBITION OF RHIZOBIA BY GERMINATING SEEDS

A paradox seen during the study was the inhibition of rhizobia not only by the germinating non-legume seeds but also by germinating legume seeds. It was observed that the germinating seeds of both legumes and non-legumes inhibit rhizobia, though the non-legume dicot seeds of sunflower and cotton inhibited the most. Toxicity of seed coat exudates to rhizobia has been reported earlier (Thompson, 1960; Young

and Peterson, 1980). It was also observed that the inhibitory action was not related to the size of the seeds as even small seeds like ragi had inhibition zones comparable to other large seeded legumes and non-legumes. Such an inhibition of rhizobia by legumes was not found to be related to any cross inoculation groups, as all the rhizobia were inhibited by all the germinating legume seeds.

Such inhibition of rhizobia was also reported for Bengal gram, green gram, soybean, leucerne, cowpea, black gram, red gram, clover, horse gram, sesbania, lupin and lima bean (Bowen, 1961; Dadarwal and Sen, 1973; Kandaswamy et al., 1974; Jain and Rewari, 1976; Balasubramanian et al., 1991; Ali and Loynachan, 1990; Abdel Nasser et al., 1986). Toxicity of non-nodulating plants in Caesalpinoideae was also reported by Rao et al. (1973). These studies indicate that the seeds of both legumes and non-legumes exude toxic substances during germination. It has been suggested that inhibition of microorganisms might be a survival phenomenon that has been evolved to prevent microbial degradation of the nutrient rich embryo as the seed starts to germinate.

The inhibition of rhizobia by germinating seeds may have practical significance during the process of seed inoculation of biofertilizers. The toxic exudation is thought to be one of the reasons for the poor survival of

the rhizobia inoculated to the seed. So it can be suggested that resistance to the germinating seed diffusates of legumes and non-legumes may partially determine the success of a Rhizobium strain inoculated into the soil. New methods of legume inoculation like use of liquid inoculant, soil inoculant, granular inoculant may help to solve the problem of seed coat toxicity.

The present study has thrown some new lights on the question of legume-Rhizobium specificity, chemotaxis and on effects of seed leacheates and root exudates of non-legume and non-host legumes on rhizobial growth and chemotaxis which needs to be further investigated.

SUMMARY

VI. SUMMARY

The effects of seed leacheate, germinating seed exudate and seedling root exudate compounds of legumes (chickpea, cowpea, soybean and groundnut) and non-legumes (maize, ragi, cotton and sunflower) on the growth and chemotaxis of rhizobia were investigated. The rhizobial strains used for the study were IC 59 (a chickpea specific strain), TAL 102 (a soybean specific strain) and P 132 and TAL 1000 strain nodulating cowpea and groundnut.

All the test rhizobia showed good growth in the root exudates of both legumes and non-legumes, indicating the absence of any preferential nourishment of rhizobia in the legume root exudates or conversely indicating the absence of any inhibitory substances to rhizobia in the non-legume root exudates tested. The study also showed no specific stimulation by legumes in promoting growth of its homologous rhizobia capable of inducing nodulation.

The chemotaxis of rhizobia towards seed leacheates and root exudates were assayed by modified Alder's capillary tube method. The rhizobia studied were all found to be chemotactic towards the seed leacheates and root exudates of all the legumes and non-legumes which were tested. It was thus concluded that although positive chemotaxis of rhizobia

to the exudates exists, it has little or no role in the symbiont specificity.

Assays determining the amino acid composition of the seed leacheates and root exudates revealed that the legume and the non-legume exudates were similar qualitatively, thus suggesting the involvement of other important compounds like the flavonoids in the initiation of the legume root nodule by the rhizobia.

Agar plate bioassay on inhibition of rhizobia by germinating legume and non-legume seeds revealed that rhizobial inhibitors were found to be released from the germinating seeds of both legumes and non-legumes. Toxic factors from germinating seeds may influence the rhizobial proliferation around the seed, especially the seed inoculated rhizobia and it may be expected to influence the rhizobial composition in the early rhizosphere.

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* Originals not seen.

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