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Facultative symbiotrophic nitrogen-fixing associations in rice soils of India

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Abstract. The occurrence of facultative symbiotrophic N_2-fixing associations in three rice soils of India is reported. Considerable variation in N_2-fixing efficiency of these associations was noticed among the soil types studied. Associations from rice straw-amended alluvial soil under both flooded and non-flooded conditions exhibited higher N_2-fixing efficiency than those from unamended soils of both water regimes. Despite high salinity and acidity an acid sulphate soil harboured N_2-fixing symbiotrophic organisms with appreciable efficiency. Application of rice straw to the soil under both flooded and non-flooded conditions stimulated N_2-fixation in alluvial, laterite and acid sulphate saline soils. These observations suggested the significant contribution of these associations to the nitrogen economy of different soil types.

Keywords. Rice soils; facultative symbiotrophic associations; acid sulphate soil; nitrogen fixation; nitrogen-fixing efficiency.

1. Introduction

The nitrogen fixation in rice soils under tropical conditions is of great significance in view of high fertilizer costs. The importance of facultative symbiotrophic nitrogen-fixing organisms in the ecosystems has been recognised recently (Jurgensen and Davey 1970; Jensen and Holm 1975). Organisms belonging to this group have a limited ability for N_2 fixation in pure culture, but can readily fix N_2 in mixed cultures with other bacteria (Kalininskaya 1967a). Although many investigations have been made on the single cultures isolated and inoculated to rice soils under Indian conditions (Purushothaman *et al* 1976; Rangaswami and Venkatesan 1966), data on the participation of symbiotrophic N_2-fixers are rather limited. It has been established that applications of rice straw to flooded paddy soil enhanced the population of N_2-fixing Azotobacter and anaerobic organisms (Rao *et al* 1973; Rao 1977). We report the occurrence of facultative symbiotrophic N_2-fixing associations and their N_2-fixing efficiency as influenced by rice straw amendment in three Indian rice soils under flooded and non-flooded conditions.

2. Materials and methods

2.1. *Soils*

Alluvial (pH 6·2, organic matter 1·6%, total N 0·09%, electrical conductivity 0·6 mmhos/cm), laterite (pH 5·0, organic matter 3·25%, total N 0·09%, electrical

conductivity 0·2 mmhos/cm), and an acid sulphate saline soil from Kerala, locally known as *Pokkali* (pH 4·2, organic matter 8·21%, total N 0·24%, electrical conductivity 8·5 mmhos/cm) were used in the study.

2.2. *Effect of rice straw on N_2-fixation in soil*

The effect of rice straw on N_2 fixation was studied by amending soils (5 g) contained in small glass vials with 0·5% (w/w) powdered rice straw and subsequent incubation for 100 days under flooded (with 7·5 ml distilled water to provide 1·5 cm standing water column) and non-flooded (50% water holding capacity) conditions. Moisture content was adjusted at regular intervals. All treatments were duplicated. The relative efficiency of facultative symbiotrophic N_2 fixers in these soils after 40 days of appropriate treatments was determined by transferring 1 ml each of serial dilutions of soil to the specific medium described under section 2.3. The total N in the soil samples was also estimated by Kjeldahl method at 0, 40 and 100 days of incubation at 28° ± 2° C.

2.3. *Symbiotrophic organisms*

Symbiotrophic N_2-fixing organisms were grown from soils incubated for 40 days under different treatments indicated in section 2.2. For enumerating the number of facultative symbiotrophic organisms, 1 ml of serially diluted (10^{-2} to 10^{-6}) soil sample was inoculated to 100 ml Erlenmeyer flasks containing 30 ml sterile N-free medium (g/l); K_2HPO_4, 1·74; KH_2PO_4, 0·91; $MgSO_4 \cdot 7H_2O$, 0·03; $CaCl_2 \cdot 6H_2O$, 0·1; NaCl, 0·5; $FeCl_3 \cdot 6H_2O$, 0·01; glucose, 10; bromothymol blue, 0·01; yeast extract 0·05; micronutrients 1 ml [containing (g/l) H_3BO_3, 0.5; $MnSO_4 \cdot 7H_2O$, 0·05; $ZnSO_4 \cdot 7H_2O$, 0·05; $CuSO_4 \cdot 7H_2O$, 0·05; MoO_3, 0·01; $CoCl_2$, 0·04] and incubated at 28°C for 30 days (Kalininskaya 1967a). This medium developed by Kalininskaya, is highly specific for symbiotrophic N_2-fixing associations. The medium, initially neutral, became acidic following glucose utilisation and the pH of the medium during incubation was maintained at 7·0 by addition of sterile dilute NaOH. Depletion of glucose was indicated by the absence of change in the pH value. At the end of the incubation, the total N (Kjeldahl) was determined in duplicate for all the serial dilutions of the soil. The increase, greater than 0·35 mg N over the N value for the uninoculated control was considered as positive fixation.

3. Results and discussions

3.1. *Assessment of nitrogen fixation in rice soils*

An assessment of the N_2 fixation was made in the alluvial, laterite and acid sulphate-saline soils following rice straw application under flooded and non-flooded conditions at 40 and 100-day incubation (table 1). Results indicate that there was considerable variation in the amount of N_2 fixed with respect to the soil type. Despite high salinity and acidity, an acid sulphate soil exhibited highest N_2-fixing ability among the soils under both flooded and non-flooded conditions. Moreover, rice straw amendment to this soil greatly stimulated N_2 fixation. Prolonged incubation (100 days)

under flooded conditions appreciably enhanced the N_2 fixation with the exception of an alluvial soil, where only rice straw amendment resulted in considerable fixation. The absence of N_2 fixation under non-flooded conditions in laterite and alluvial soils at 100 days could probably be due to the inadequate moisture level and nutrient availability for effective N_2 fixation. Rice straw amendment to flooded soils protects the inorganic N from nitrification and subsequent denitrification by immobilisation (Patrick and Gotoh 1974). Evidently, the enhanced N_2 fixation combined with reduced N loss by immobilisation, in rice straw-amended soils, has led to overall N gain even after 100-day incubation. In fact, rice straw additions to both moist and flooded soils enhanced N_2 fixation (Rao 1976).

3.2. *Nitrogen fixation by symbiotrophic associations*

Facultative symbiotrophic N_2-fixing organisms have complex nutritional requirements for their growth and N_2 fixation, both of which are achieved in association with a non-N_2 fixer and are widespread in most soil types. Moreover, these associations are non-specific in nature (Kalininskaya 1967b).

The facultative symbiotrophic associations obtained from rice straw-amended alluvial soil under two water regimes exhibited greater N_2-fixing efficiency as compared to the associations from corresponding unamended soil (table 2). Conversely, associations from a non-flooded laterite soil were more efficient than associations

Table 1. Nitrogen fixation as influenced by rice straw and moisture regime in rice soils of India.

Soil type	Nitrogen fixed (μg/g soil)			
	Incubation (days)			
	40		100	
	Non-flooded condition	Flooded condition	Non-flooded condition	Flooded condition
Alluvial	182 ± 2·8	56 ± 4·2	0·0	0·0
Alluvial + RS	308 ± 5·4	174 ± 10·2	0·0	200 ± 39·2
Laterite	92 ± 5·6	92 ± 9·8	0·0	348 ± 23·8
Laterite + RS	274 ± 2·8	240 ± 12·6	110 ± 5·6	526 ± 14·0
Acid sulphate, saline	238 ± 45·0	128 ± 4·2	564 ± 72·8	388 ± 18·2
Acid sulphate, saline + RS	330	274	572 ± 36·4	740 ± 12·6

RS = rice straw.

Table 2. Nitrogen-fixing efficiency of facultative symbiotrophic associations from rice soils

Soil type	Nitrogen-fixing efficiency (N fixed mg/g glucose)			
	Associations from:			
	Flooded soil		Non-flooded soil	
	Unamended	Rice straw amended	Unamended	Rice straw amended
Alluvial	2·2 ± 0·17	5·9 ± 1·60	3·8 ± 0·15	5·6 ± 0·80
Laterite	3·1 ± 0·32	3·4 ± 0·40	3·9 ± 0·37	4·2 ± 0·08
Acid sulphate saline	2·8 ± 0·35	3·6 ± 0·40	1·8 ± 0·08	2·7 ± 0·30

from flooded, unamended and rice straw-amended soils with respect to N_2 fixation. Despite high acidity and salinity of an acid-sulphate *Pokkali* soil, associations from this soil exhibited considerable N_2-fixing efficiency. Moreover, an appreciable increase in the N_2 fixation by these associations was noticed when the acid sulphate saline soil was flooded or amended with rice straw under both flooded and non-flooded conditions. Low N_2-fixing efficiency of the associations from non-flooded *Pokkali* soil could be ascribed, in part, to low pH, whereas flooding had no adverse effect on N_2 fixation since, following few days after flooding, the pH of this soil attained near neutrality (Siddaramappa and Sethunathan 1975). Preliminary morphological characterisation suggested the presence of *Pseudomonas* sp., *Mycobacterium* sp., *Arthrobacter* sp., and *Bacillus* sp., in these associations and further confirmation by biochemical means is in progress.

These results reveal the occurrence of active facultative symbiotrophic N_2-fixing organisms in rice soils and their overall contribution to the N_2 fixation in soils of different types. Further, these studies emphasise the necessity of monitoring the activities of organisms following judicious use of amendments for exploiting their maximum efficiency in soils under rice cultivation.

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References

Jensen V and Holm E 1975 in *Nitrogen Fixation by Free-living Micro-organisms* ed W D P Stewart (Cambridge: Cambridge University Press) p 101

Jurgensen M F and Davey C B 1970 *Soils Fert.* **33** 435

Kalininskaya T A 1967a *Mikrobiologiya* **36** 436

Kalininskaya T A 1967b *Mikrobiologiya* **36** 520

Patrick W H Jr and Gotoh S 1974 *Soil Sci.* **118** 78

Purushothaman D, Obilisami G and Balasundaram C S 1976 *Madras Agric. J.* **63** 595

Rangaswami G and Venkatesan R 1966 in *Micro-organisms in Paddy Soil* (Madras: Annamalai University Press)

Rao V R, Kalininskaya T A and Miller U M 1973 *Mikrobiologiya* **42** 729

Rao V R 1976 *Soil Biol. Biochem.* **8** 445

Rao V R 1977 *Curr. Sci.* **46** 118

Siddaramappa R and Sethunathan N 1975 *Pestic. Sci.* **6** 395