Bean Nodulation Patterns in Soils of Different Texture at Morogoro, Tanzania

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Abstract

*This study was designed to examine the relationship between nodulation in a bean-**Rhizobium** system in three soils of varying texture and % recovery of rhizobial cells immediately after inoculation into such soils. Effects of inoculation methods (seed pelleting versus soil inoculation) on nodulation and plant growth were investigated in the three soils using a serologically-marked **Rhizobium** strain (CIAT 899) which was subsequently monitored in the nodules by the enzyme-linked immunosorbent assay (ELISA) technique. When the major soil chemical factors affecting nodulation were standardized, bean nodulation patterns continued to be closely related to soil texture, being higher in the sandy and loamy soils than in the fine-textured clay. Soil inoculation consistently gave better nodulation than seed pelleting. According to the ELISA procedure however, percent nodule occupancy by CIAT 899 showed patterns completely the opposite of the nodulation trends outlined above, being 100% in the clay but only 10% in the sandy soil. Thus, nodulation success by the inoculum was total in the clay but only dismal in the sandy soil. The unexpected discrepancy between inoculum success on the one hand and nodulation plus plant growth response on the other, is discussed.*

Keywords: Bean nodulation, ELISA typing of nodules, *phaseolus vulgaris*

Introduction

The response of beans (*Phaseolus vulgaris*) to *Rhizobium* inoculation in many parts of the world (Tanzania inclusive) is very inconsistent (Graham and Temple, 1984). Apart from soil chemical or biotic stresses, other soil factors not considered as limiting before, could account for unsatisfactory legume inoculation responses. The physical interaction of *Rhizobium* inoculum cells with the soil matrix, as a possible cause of inoculation failure, has not been critically examined, despite the generally accepted fact that when bacteria are added to soils, they are generally sorbed by physical and weak electrostatic forces to the soil colloidal particles (Marshall, 1980). Msumali and Mirisho (1988) estimated this bacterial (*Rhizobium*) cell adsorption indirectly by determining % cell recovery (i.e. % of the cells recovered by plate counts, only 30 minutes after inoculation) in sandy, sandy clay loam (subsequently referred to simply as "loam") and clay soils. In that study, % cell recovery was highest in the sandy, intermediate in the loam and lowest in the clay soil. Factors known to affect rhizobial viability had been standardized

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in the three soils and, no toxic factors were detected in the clay soil from which the lowest cell recovery was recorded. The low recovery was therefore presumed to have been caused by an irreversible attachment of cells to soil particles. A further study by Msumali and Mirisho (1988) indicated a close correlation between nodule number and % cell recovery in bean, pea and cowpea plants sown in the 3 soil types, and % cell recovery of rhizobia inoculated into such soils. In the present study, the above phenomenon was further examined using natural (non-sterile) soil in which beans were inoculated with a serologically-marked strain of *Rhizobium*.

Materials and Methods

Materials

Soils: The soils, sampled from three different locations within the Sokoine University of Agriculture (SUA) farm, had wide variations in texture and in chemical properties (notably pH and available nutrients -Table 1). This variation necessitated a standardization (described later) in the chemical properties known to affect nodulation, prior to the initiation of the main study.

Bean variety and the Rhizobium culture: A bean variety TMO 216 was obtained from the Department of Crop Science and Production at SUA. A peat-based culture of *Rhizobium leguminosarum* bv *phaseoli* strain CIAT 899 was supplied by the CIAT Microbiology Laboratory in Cali, Colombia. At the time of initiating the study, it contained 2.3 x 10^8 viable cells/g. This rhizobial culture has since been renamed *Rhizobium tropici* (Martinez *et al.*, 1991).

Methodology

Adjustment of soil chemical properties

The loam soil was amended with 1 m.e. of $CaCO_3$/100 g soil and incubated at field capacity moisture content for 2 weeks. This treatment raised the soil pH to 6.1 (common with the sandy and clay soils - Table 1). The loam and clay soils were brought to the same P level as the sandy soils by adding 1.125 or 1.145 g portions of ground and well-mixed samples of triplesuperphosphate per 500 g of the loam and clay soils respectively. Available N in each of the soil, although relatively low at the start, was further depleted by biological immobilization through the incorporation of ground, N - poor maize straw (C:N ratio 60:1). At an application rate of 0.25% w/w (field equivalent of 5 tonnes/ha) the available N

Table 1: Initial properties of the soils used in the bean nodulation-patterns study

Sampling site	Soil type	pH and available nutrients (-kg/ha)		
		pH	N	P
Horticultural Unit	Sandy	6.1	17.5	93.4
Close to Univ. gate	Loam*	5.4	24.5	2.8
Magadu	Clay	6.1	10.0	1.4

*The actual texture is a sandy clay loam in all the soils was reduced to very low levels (less than 1 ppm) after two weeks of incubation at field capacity moisture content. After the necessary adjustments, the soils were transferred in 500g amounts, into Leonard jars.

Seed inoculation, planting and the experimental design

Seed pelleting (SP): A water slurry of 1 g of the peat culture was pelleted on 27 seeds of TMO 216. This rate of inoculation gave a theoretical inoculum rate of 8.5 x 10^6 cells/seed. Three seeds were planted and later thinned to 2 plants per jar. Inoculated seeds were planted at a depth of ca. 2 cm below the surface and covered with soil.

Soil inoculation (SI): An approach was used to avoid varying the final cell number introduced into the soil by the two inoculation methods so as to permit a valid interpretation of the effects of the inoculation methods. Thus, as the inoculation rate in the SP method provided 8.5 x 10^6 cells per planting hole, approximately 0.04 g of the inoculum was placed in each of the 3 planting holes ca. 2 cm deep, and 0.2 ml water was added. Seed was then placed on top of the inoculum and covered with soil.

Controls: Uninoculated controls **(Io)** had seeds planted in each of the soils without *Rhizobium* inoculation.

Each of the above treatments was replicated 3 times and the jars were arranged in a randomized complete block design, under glass-house conditions. Data collected 35 days after planting included nodule count, plant (shoot) fresh weight and N content (%). The nodules were later Atyped@ to determine percentage of inoculant strain nodule occupancy, using the enzyme-linked immunosorbent assay (ELISA) technique (Kishinevsky and Gareth-Jones 1987). Since the nodulation pattern was strongly influenced by soil texture (Table 2), only the extreme soil textural classes (i.e sandy and clay) were examined for their effects on % nodule occupancy by the inoculum.

Results and Discussion

The effect of soil texture, averaged across inoculation methods (Table 2) revealed a declining trend in nodulation from the sandy though loam to the clay soils. Soil inoculation gave a significantly higher number of nodules per plant than seed pelleting. Significantly higher shoot weights were obtained in the sandy and loam soils than in the clay soil. Soil inoculation gave significantly higher shoot weight than seed pelleting (Table 2). However, the beneficial effect of soil inoculation was significant only in the loam soil but not in the sandy or clay soils. Soil type and inoculation methods did not have statistically significant effects on shoot N content (Table 2)

The nodulation trends in soils of different texture are consistent with the observations made earlier by Msumali and Mirisho (1988) in which, shoot weight was consistent with the nodulation patterns. Since the major soil chemical properties likely to affect nodulation were standardized, soil texture appears to be the major variable accounting for these differences. However, if the "cell adsorption" proposition by Msumali and Mirisho (1988), accounting for differences in nodulation is to be upheld, then we should expect that soil inoculation, which results in better distribution of the inoculum cells within the soil, and therefore, independent of inoculum "mobility", to result in better nodulation and plant growth. This appears to have been the case in the study by Wadisirisuk *et al* (1989) and that of Msumali and Mirisho (1988) in which the soil inoculation was achieved by complete mixing of the inoculum with the soil. In the present study however, the soil inocu-

Table 2: Effects of soil texture and method of inoculation (with *Rhizobium tropici*) on the nodulation, shoot weight and N content of bean (*Phaseolus vulgaris*) cv TMO 216 at Morogoro

Parameter	Innoculation method	Soil type			Mean	SE	CV (%)
		Sandy	Loam	Clay			
(a) Nodulation (no./plant)	SI	72	131	49	84		
	SP	61	12	18	30		
	I_0	32	15	21	23	19 (***)	
	Mean	55	53	29			88.4
	SE		(19 NS)				
(b) Shoot fresh weight (g/plant)	SI	6.43	10.55	4.61	7.20		
	SP	4.60	3.18	3.34	3.72		
	I_0	6.88	3.80	0.96	4.65	0.58 (***)	
	Mean	5.99	5.68	3.91			
	SE		0.58 (**)				23.7
(c) Shoot N content (%)	SI	1.01	1.38	1.34	1.24		
	SP	0.98	1.13	0.96	1.03		
	I_0	1.16	1.22	0.82	1.06	0.10 (NS)	
	Mean	1.05	1.28	1.04			
	SE		(0.10 NS)				19.8

(1) *, ** and *** indicate significant differences at the 5, 1 and 0.1% probability levels, respectively.

(2) NS indicate non-significant differences.

(3) The CV values were the combined effect of soil types and inoculation methods.

lation treatment involved placing the inoculum on one spot beneath the seed. Therefore, the better nodulation and plant growth when the inoculum was not seed-applied cannot wholly be explained in terms of better inoculum distribution due to placement.

Nodule occupancy by the inoculum strain in the sandy and clay soils, as determined by the ELISA technique, gave results which seem to contradict rather than complement the above interpretations. Table 3 shows that % nodule occupancy values were low (0-10%) in the sandy soil but high (90-100%) in the clay soil, indicating that the plants were almost completely nodulated by the inoculum strain in the clay soil while they were almost completely nodulated by the native strains in the sandy soil. Yet, it was in the sandy soil in which nodulation and plant growth were better than in the clay soil. It is not clear as to why, when the inoculum was most successful in nodulation (in the clay soil), this did not result in a corresponding appreciable response in nodule numbers and plant growth.

Table 3: Percent nodule occupancy of inoculum strain CIAT 899 as affected by soil texture and inoculation methods. (Data based on ELISAtyping of the nodules)

Soil texture	Inoculation method	% Nodule occupancy by inoculum strain
Sandy	SI	0
	SP	10
	I_0	0
Clay	SI	100
	SP	90
	I_0	0

Note: SI = Soil inoculation; SP = Seed pelleting; I_0 = Uninoculated control

Conclusion

The ELISA data failed to support the cell adsorption hypothesis as a factor accounting for nodulation differences in soils of different texture. But the same data also failed to support the generally-held contention that competition for host nodulation is an overriding factor limiting bean response to *Rhizobium* inoculation. The importance of gaining better nodule occupancy by selected strains for improved overall plant performance clearly requires that the inoculum strain be significantly more effective than the native population.

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