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EFFECT OF *RHIZOBIUM* NUMBERS ON NODULATION AND DINITROGEN FIXATION IN GROUNDNUT†

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SUMMARY

Glasshouse studies have shown that nodulation and dinitrogen fixation in groundnuts grown in a sand/vermiculite mixture increased with an increase in *Rhizobium* numbers inoculated onto each seed. Cultivars Robut 33-1 and TMV 2 required 10^6 rhizobia seed^{-1} for maximum nodulation and dinitrogen fixation, while cv. Kadiri 71-1 required larger numbers.

Groundnut (*Arachis hypogaea* L.) yields can be increased by inoculating crops with efficient and competitive strains of *Rhizobium* (Schiffman and Alper, 1968; Burton 1976a; Nambiar *et al.*, 1981). Groundnuts are nodulated by a group of rhizobia classified as 'cowpea miscellany' (Fred *et al.*, 1932) and there are large differences in the dinitrogen fixing efficiency of these strains (Allen and Allen, 1940; Weaver, 1974; Wynne *et al.*, 1980; Nambiar and Dart, 1980).

Strains of *Rhizobium* should be screened initially under controlled environment conditions for their effectiveness in dinitrogen fixation before being recommended for field inoculation trials (Date, 1976; Elkan *et al.*, 1980). Relative effectiveness is quite often tested by comparing the nitrogen (N) accumulated by plants grown without N but inoculated with the desired strain with that gained by non-nodulated plants given inorganic-N. Reports indicate that legume species differ in the number of rhizobia required for maximum nodulation and dinitrogen fixation. In soyabeans, some cultivars were reported to require only 50–100 rhizobia seed^{-1} (Perkins, 1925; van Rensburg and Strijdom, 1969) whereas others need more than 1000 rhizobia seed^{-1} (Weaver and Frederick, 1972). Bhaduri (1951) observed that the number of nodules formed on the roots of *Phaseolus radiatus* (now *Vigna radiata*; Verdcourt, 1980) grown in water culture was increased only moderately by a large increase in numbers of *Rhizobium*. Based on their observations on clovers, Purchase and Nutman (1957) concluded that 10^3–10^4 rhizobia in the root rhizosphere were adequate to produce maximum numbers of nodules. This paper reports the effects of applying different numbers of *Rhizobium* on nodulation and dinitrogen fixation in groundnuts grown under controlled conditions.

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MATERIALS AND METHODS

Cultivars and strains

Three cultivars were used: Kadiri 71-1 (Virginia Runner), Robut 33-1 (Virginia Bunch) and TMV 2 (Spanish Bunch). The strains of *Rhizobium* were NC 92 (obtained from Dr G. H. Elkan, North Carolina State University, USA) and 5a/70 (from Dr Rina Lobel, The Volcani Center, Israel). Peat cultures of each strain were prepared as described by Thompson (1980) and diluted with sterile water to obtain the desired numbers.

Plant growth conditions

Since the conventional Leonard jar system had failed to produce good plant growth, a pot culture system incorporating free-drainage and precautions to control sterility was used. The details of this system have been described by Nambiar and Dart (1980). Plants were grown in a sterilized sand/vermiculite mixture (2:1 v/v). The seeds were sterilized with aqueous mercuric chloride (0.1%) for 5 min and were washed ten times with sterile water before sowing. Eight seeds were sown into each pot and thinned to three soon after emergence (5-6 days after sowing). After thinning, seedlings were inoculated with the appropriate strain of *Rhizobium* at the desired population in 1 ml water suspensions and pots were watered immediately. The pot surfaces were then covered with a layer of 5 mm gravel to reduce surface contamination and were watered through 2 cm diameter plastic pipes (Nambiar and Dart, 1980).

Plants were grown in a glasshouse at 28 ± 4°C and 23 ± 4°C day and night temperatures, respectively. They were irrigated with sterilized water (to 50-60% water holding capacity) and a sterilized nitrogen-free nutrient solution containing 1 mM $CaCl_2$, 0.5 mM KH_2PO_4, 10 μM $(CH_2.N\ (CH_2.COO)_2)_2$ FeNa, 0.25 mM $MgSO_4$, 0.25 mM K_2SO_4, 1 μM $MnSO_4\ 4H_2O$, 2 μM H_3BO_3, 0.5 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.1 μM $CoSO_4$ and 0.1 μM Na_2MO_4, was supplied on alternate days (Broughton and Dilworth, 1971). At seven day intervals pots were flushed with 1.5 l deionized, sterilized water to prevent any accumulation of nutrient salts, followed by 100 ml of sterile nutrient solution. The pots were arranged in a randomized block design with five blocks.

Two experiments were conducted. In the first one, cv. Kadiri 71-1 was inoculated with strain NC 92 at four inoculum densities (10^2, 10^4, 10^7 and 10^9 rhizobia seed^{-1}). In the second, cvs TMV 2 and Robut 33-1 were inoculated with strain NC 92 or 5a/70 at four inoculum densities (10^2, 10^4, 10^6 and 10^8 rhizobia seed^{-1}). Plants were harvested at 60 days after planting when shoot and nodule weights were recorded after drying at 80°C for 24 h and N contents in plant tissues were determined (Technicon Autoanalyser 11, Industrial method Nc.218-72, AA 11). The data were transformed using logarithms to base 10 prior to analysis.

RESULTS

The response of cv. Kadiri 71-1 to different populations of the strain NC 92 is shown in Table 1. Both shoot and nodule weights increased with the increased numbers of rhizobia in the inoculum (by a factor of 3.5 and 6.5, respectively, at 10^9 compared with 10^2 rhizobia seed^{-1}).

In the second experiment, with two cultivars and two strains, the number of nodules plant^{-1} increased from 6–11 at 10^2 to 143–243 at 10^8 rhizobia seed^{-1} (Table 2). Many plants of cv. Robut 33-1 inoculated with strain NC 92 (10^8 rhizobia seed^{-1}) became infected with 'bud necrosis' disease and so this treatment was discarded. Maximum nodulation was observed on cv. TMV 2 inoculated with strain NC 92 at 10^8 rhizobia seed^{-1}. Very few nodules were formed on the primary root when only 10^2 rhizobia seed^{-1} were used (Fig. 1; Table 3). Nodule weight also increased with an increase in inoculum density (Table 4).

The effect of increasing *Rhizobium* numbers on dinitrogen fixation is shown in Tables 1 and 5. The Virginia cultivar, Kadiri 71-1, required a larger number

Table 1. *Influence of* Rhizobium *inoculum density on nodulation and N_2-fixation by groundnut cv. Kadiri 71-1*

Inoculum density (rhizobia seed^{-1})	Shoot dry wt (g plant^{-1})	Nodule dry wt (mg plant^{-1})	Total nitrogen content (mg plant^{-1})
6.1×10^2	0.97	20 (1.13)	14.3 (1.16)
4.8×10^4	1.08	30 (1.39)	18.6 (1.13)
5.5×10^7	2.38	120 (2.05)	50.3 (1.69)
3.2×10^9	3.38	130 (2.10)	73.2 (1.86)
Nitrate control†	4.34	0	89.8 (1.93)
SED	0.558	(0.34)	(0.142)

† Nutrient solution supplemented with 250 ppm N (as potassium nitrate).
Log_{10} transformed means are given in parentheses.

Table 2. *Effect of* Rhizobium *number on nodulation (no. nodules plant^{-1})*

	Cultivar			
	Robut 33-1		TMV 2	
Inoculum density (rhizobia seed^{-1})	Strain 5a/70	Strain NC 92	Strain 5a/70	Strain NC 92
2.7×10^2	11 (0.998)	9 (0.902)	7 (0.741)	6 (0.619)
2.7×10^4	50 (1.329)	18 (1.229)	46 (1.338)	57 (1.732)
2.7×10^6	118 (2.034)	131 (2.093)	129 (2.103)	182 (2.248)
2.7×10^8	160 (2.141)	–	143 (2.128)	243 (2.371)

Log_{10} transformed means are given in parentheses. The standard error for comparing differences between any transformed means is 0.1786.

Table 3. *Effect of* Rhizobium *numbers on nodulation on the primary root (no. nodules plant^{-1})*

Inoculum density (rhizobia seed^{-1})	Cultivar: Robut 33-1, Strain 5a/70	Cultivar: Robut 33-1, Strain NC 92	Cultivar: TMV 2, Strain 5a/70	Cultivar: TMV 2, Strain NC 92
2.7×10^2	0.8 (0.195)	1.0 (0.269)	2.8 (0.270)	1.5 (0.294)
2.7×10^4	1.5 (0.325)	5.5 (0.736)	4.5 (0.634)	13 (0.958)
2.7×10^6	24 (1.350)	22 (1.254)	30 (1.256)	26 (1.152)
2.7×10^8	67 (1.405)	–	78 (1.3826)	166 (2.207)

$1+\log_{10}$ transformed means are given in parentheses. The standard error for comparing differences between any transformed means is 0.2675.

Table 4. *Effect of* Rhizobium *numbers on nodule weight (mg plant^{-1})*

Inoculum density (rhizobia seed^{-1})	Cultivar: Robut 33-1, Strain 5a/70	Cultivar: Robut 33-1, Strain NC 92	Cultivar: TMV 2, Strain 5a/70	Cultivar: TMV 2, Strain NC 92
2.7×10^2	25 (1.358)	18 (1.248)	18 (1.134)	18 (1.225)
2.7×10^4	53 (1.538)	36 (1.513)	45 (1.428)	79 (1.889)
2.7×10^6	109 (2.035)	125 (2.080)	110 (2.029)	101 (1.998)
2.7×10^8	109 (2.035)	–	104 (2.015)	119 (2.068)

Log_{10} transformed means are given in parentheses. The standard error for comparing differences between any transformed mean is 0.1389.

Table 5. *Effect of* Rhizobium *numbers on dinitrogen fixation (total nitrogen content; mg plant^{-1})*

Inoculum density (rhizobia seed^{-1})	Cultivar: Robut 33-1, Strain 5a/70	Cultivar: Robut 33-1, Strain NC 92	Cultivar: TMV 2, Strain 5a/70	Cultivar: TMV 2, Strain NC 92
2.7×10^2	23.3 (1.336)	24.8 (1.365)	19.4 (1.281)	16.5 (1.203)
2.7×10^4	40.9 (1.545)	22.5 (1.334)	28.1 (1.439)	29.1 (1.446)
2.7×10^6	59.7 (1.775)	44.6 (1.639)	62.9 (1.787)	36.7 (1.547)
2.7×10^8	61.4 (1.787)	–	63.5 (1.793)	45.8 (1.650)

Log_{10} transformed means are given in parentheses. The standard error for comparing differences between any two transformed means is 0.1028.

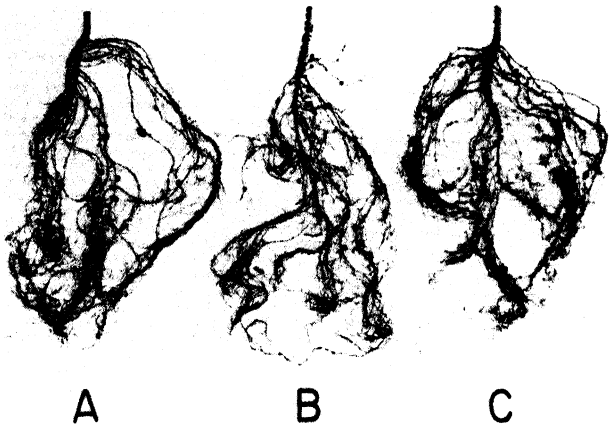

Fig. 1. Effect of *Rhizobium* density on nodulation of cv. Kadiri 71-1 (A, B and C denote 10^2, 10^4 and 10^8 rhizobia seed^{-1}, respectively).

of rhizobia for maximum dinitrogen fixation, with increases up to 10^9 rhizobia seed^{-1} (Table 1). However, with cvs TMV 2 and Robut 33-1 the nitrogen fixed at 10^6 rhizobia seed^{-1} was close to that at 10^8 (Table 5). A linear regression analysis for total nitrogen in the plant *v.* nodule weight and nodule number is shown in Figs 2 and 3. The regression was significant ($P < 0.01$) in all cultivar x strain combinations.

DISCUSSION

In all three cultivars, both nodulation and dinitrogen fixation increased with an increase in *Rhizobium* numbers; up to a maximum of 10^6 rhizobia seed^{-1} for TMV 2 and Robut 33-1 and up to 10^9 seed^{-1} in cv. Kadiri 71-1. Burton (1976b) reported that 10^4 rhizobia seed^{-1} were adequate for maximum dinitrogen fixation in soyabean cv. Traverse whereas van Rensburg and Strijdom (1969) did not observe any significant differences in plant response to increased inoculum density from 10^2 to 10^8 rhizobia seed^{-1} in three other soyabeans. Weaver and Frederick (1972) discovered that the growth medium influences the population required for maximum nodulation and dinitrogen fixation in soyabean cv. Ford. Maximum dinitrogen fixation was achieved by 2.4×10^3 rhizobia seed^{-1} in soil-grown plants while 100,000 rhizobia were required for those grown in sand. We have obtained the best plant growth and nodulation in a sand/vermiculite mixture. Data indicate that multiplication of rhizobia in this medium is not adequate to compensate for initial differences in inoculum rate. Therefore, unless the inoculum densities of different strains are large and constant, it is likely that variable results will be obtained in screens for symbiotic efficiency.

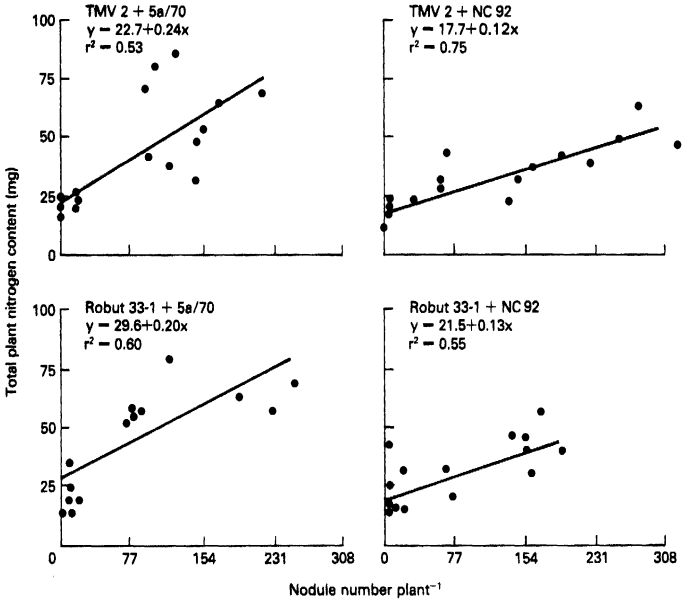

Fig. 2

Figs 2 and 3. Regression analyses showing N_2-fixing efficiency of two *Rhizobium* strains (NC 92 and 5a/70) on two groundnut cultivars (TMV 2 and Robut 33-1).

Variations in nodule weight and nodule number for plants inoculated with a given number of rhizobia seed^{-1} provided data for a linear regression analysis relating these attributes to the amount of N_2-fixed (Figs 2 and 3). The slopes of the regression lines were more-or-less identical for both cultivars inoculated with a given strain. Strain 5a/70 fixed more dinitrogen than NC 92 at a given inoculum density and especially so at the largest inoculum rates (Table 5). Strain NC 92 formed more nodules with the large inocula but fixed less dinitrogen than strain 5a/70. For example, 100 nodules of 5a/70 fixed about 50 mg N on cvs TMV 2 and Robut 33-1 whereas the same number of nodules formed by NC 92 fixed only 28–32 mg N. Strain 5a/70 increased pod yields of cv. Robut 33-1 in only one of seven field trials whereas strain NC 92 increased yields in five out of eight trials (Nambiar and Dart, unpublished results). This suggests that strain NC 92 may be more competitive than 5a/70 under field conditions.

In field soil, where inoculated strains have to compete with the native *Rhizobium* population, the numbers of rhizobia required to produce maximum nodulation are likely to be larger than those needed under glasshouse conditions.

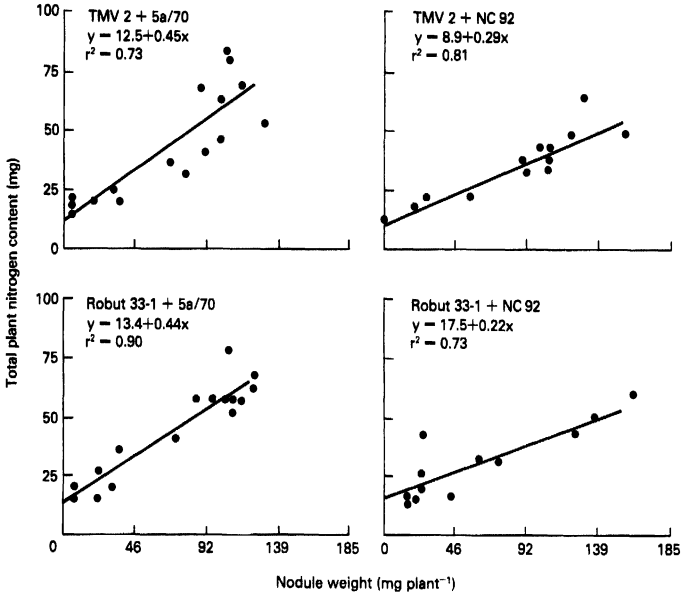

Fig. 3

Weaver and Frederick (1974) postulated that nodulation of soyabeans planted in soils containing 10^4, or more, effective rhizobia g soil^{-1} is unlikely to be increased by inoculation. We are now testing the numbers of rhizobia required for effective nodulation of groundnuts by the desired strain under field conditions with different indigenous populations of rhizobia in the soil.

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