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Greenhouse and field evaluation of *Rhizobium* strains nodulating groundnut (*Arachis hypogaea L.*)

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Nineteen *Rhizobium* isolates were screened for effectiveness in nitrogen fixation with groundnut *cv.* Virginia Florigiant. Strains exhibited wide variation in effectiveness. Five strains were marked with either streptomycin or kanamycin and used as inoculants in field studies. Assessment of strain competitive ability was complicated by isolation of kanamycin-resistant strains from uninoculated plots. Inoculation appeared to increase groundnut yields.

Keywords: *Arachis hypogaea* L.; *Rhizobium*; Yield

Remarkable increases in groundnut yields have been achieved through inoculation with *Rhizobium* (Cobb & Whitty, 1973; Stoddard, 1978). This has, however, occurred mainly in areas which were cultivated to groundnuts for the first time. Generally, groundnuts have been found to nodulate freely and effectively with a wide range of cowpea-type *Rhizobium* strains (Dart, 1974) and indications are that up to 80% of the plant's N needs can be obtained through nitrogen fixation (Dart & Krantz, 1976).

Nodulation of groundnut by indigenous bacteria is normally assumed to be adequate and inoculation is seldom practised. However, high soil temperatures, moisture stress, low pH, and poor fertility hamper survival and effective functioning of rhizobia (Marshall, 1964; Holding & King, 1963; Sprent, 1971; Vincent, 1965). Maximum potential of the symbiotic system therefore, may never be realized.

This study was aimed at evaluating *Rhizobium* strains for effectiveness, competitive ability for nodulation in the field, and for effect on yield of groundnut.

Materials and methods

Strain trial

Nineteen *Rhizobium* strains, five imported and 14 local isolates, were screened for effectiveness with *cv.* Virginia Florigiant in sterilized vermiculite in Leonard jars (Vincent, 1970). Each jar was supplied with N-free, half-strength Dart and Pate nutrient solution (Dart & Pate, 1959). Seed, sterilized by 4 min immersion in 0.2% acidified $HgCl_2$ and subsequent washing in sterile water, was planted aseptically in the Leonard jars. Plants were thinned to one per jar at germination and inoculated in triplicate with 1 ml of bacterial suspension. After six weeks growth, plants were harvested, and nodules and shoots dried at 80°C for 48 h and weighed. Shoots were analysed for % N by the semi-micro Kjeldahl procedure (Bremner, 1965).

Development of marked strains

Streptomycin- and kanamycin-resistant mutants of 5018, 22B, AH6, AH10b and CB756 were isolated by spreading 0.2 ml of yeast-mannitol broth (YMB) grown culture over plates of yeast-mannitol agar (YMA) containing 100 μg ml^{-1} of either streptomycin (S) or kanamycin (K). Colonies growing on the media after 14 days incubation at 28°C were selected as antibiotic-resistant strains. Colonies of 5018S, 22BK, CB756K, AH6K, and AH10bK were selected, cross-tested for resistance, checked for retention of symbiotic effectiveness, sub-cultured on YMA slopes, and stored at 4°C.

Field experimentation

The field experiment was established on Yellow Brown Association soil—a smectoid clay of pH 7.7. The area was divided into 18 plots (75 cm x 100 cm) and seed planted with 20 cm inter-row spacing and 25 cm within the row. Each plot was surrounded by two guard rows, respectively 125 cm x 150 cm and 175 cm x 200 cm, with similar plant spacing. Six treatments were imposed consisting of five strains and an uninoculated control, each replicated three times in a randomized block design. Plants were fertilized with 80 kg ha^{-1} muriate of potash.

Inoculum was prepared by streaking marked strains onto YMA flats and incubating at 28°C for 14 days. Cells were removed by shaking with sterile glass beads and 50 ml YMB. This was added to 200 ml sterile skim milk (100gl^{-1}) and stored at 4°C prior to use. For the purposes of inoculation this concentrate was diluted with water to three litres, and watered onto the seed in the planting furrow at one litre per plot. The final inoculum concentration was $\approx 1 \times 10^7$ cells ml^{-1}.

After eight weeks, two plants were removed from each plot. Five nodules were taken from around the crowns of the roots and stored over silica gel. Plant samples were dried, weighed and analysed for nitrogen. Dried nodules were rehydrated and

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Table 1 *Arachis—Rhizobium* strain trial

Rhizobium strain	Shoot % N	Shoot dry weight, g	Nodule dry weight, mg
5018	3.23 a*	2.35 cdefg	37.6
NGR 55	3.20 a	3.52 a	78.1
cpea 7D	3.14 ab	2.90 b	86.3
22 B	3.05 abc	2.82 bc	30.5
AH 6	2.96 abcd	2.64 bcd	33.0
Shaw CB 756	2.93 abcd	2.56 bcde	45.1
AH 5	2.93 abcd	2.46 cdefg	33.0
Nitrogen control	2.81 bcde	2.48 bcdef	—
AH9	2.80 cde	1.46 k	20.6
cpea 7B	2.79 cde	1.83 hijk	64.7
AH 2a	2.72 cde	2.12 efghi	41.6
AH 3a	2.66 de	1.87 hijk	43.1
CB 756	2.65 de	2.19 defgh	25.2
655/MAR	2.52 e	2.86 b	36.2
cpea 7	2.51 e	3.51 a	140.2
AH 10a	2.49 e	2.51 bcdef	43.5
AH 10b	2.13 f	2.49 bcdef	53.8
AP 108	2.07 f	1.49 k	29.7
TOB 7c	1.84 fg	1.94 ghijk	23.4
16 a	1.65 gh	2.95 b	17.8
Uninoculated control	1.55 gh	1.66 ijk	—
SE	0.12	0.17	

*Means followed by a common letter are not significantly different at the 5% level of probability as determined by Duncan's Multiple Range Test

sterilized as before and antibiotic-resistant *Rhizobium* strains were identified by aseptic maceration and streaking on YMA plates containing the appropriate antibiotic. Plates were incubated at 28°C for 7-10 days and examined for growth. At harvest, the number of plants in each plot was determined, pods were air-dried for 10 days and the weight determined. Yield was expressed as kg ha^{-1} of air-dried pods. Weight of 100 kernels, 100 pods, and shelling percentage was determined for each treatment.

Table 3 Recovery of marked strains

Rhizobium strain	Nodules typed	%Streptomycin	%Kanamycin
22 BK	15	NT*	47
AH 6K	15	NT	60
5018 S	15	0	NT
CB 756K	15	NT	53
AH 10bK	15	NT	33
Uninoculated	15	0	33

*NT not tested

Results

Strain trial

Wide variations in symbiotic effectiveness between strains are indicated (Table 1). Seven strains performed better (shoot %N) than did the mineral nitrogen control. However, CB756, recommended as an inoculant strain for groundnuts and other crops of the same cross-inoculation group (Staphorst *et al*, 1975; Elmes, 1976), was only moderately effective.

Nitrogen concentration in the shoot is perhaps the best direct measure of plant benefit from the symbiosis. Table 2 indicates that dry weight of shoots is significantly correlated with shoot %N. It is, however, a less reliable measure of symbiotic effectiveness, as it is more sensitive to variations in initial seed size or weight. %N was also significantly correlated with nodule dry weight (Table 2).

Table 2 Correlations of data from strain trial

Parameters	Correlation coefficient (*r*)	Significance level (*P*)
Shoot %N on shoot dry weight	0.54	0.01
Shoot %N on nodule dry weight	0.47	0.05
Shoot dry weight on nodule dry weight	0.65	0.01

Response to inoculation

Kanamycin-resistant strains were recovered from all plots into which they were inoculated (Table 4). However, the uninoculated plots yielded 33% kanamycin resistance from typed nodules. This hampered an accurate assessment of competitive ability for nodulation, except with 22BK which has a distinctive morphological appearance on YMA. Twenty per cent. of the nodules typed from plots inoculated with 22BK were formed by the inoculant strain. No streptomycin-resistant strains were recovered from plots inoculated with 5018S or from uninoculated plots.

The data indicate no significant differences between treatments for any of the parameters measured (Table 4). Despite this, inoculation with 22BK and AH6K increased yields by 82 and 64%, respectively. In addition, inoculated treatments gave higher values for shoot dry weight, 100 kernel weight and 100 pod weight than did the uninoculated treatment.

Discussion

Several methods have been used for assessing *Rhizobium* effectiveness, including measurement of

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Table 4 Response to inoculation

Rhizobium strain	Shoot dry weight, g	Yield, kg ha^{-1}	100 kernel weight, g	100 pod weight, g	Shelling percentage
22 BK	6.51 a*	929.1 a	95.72 a	280.93 a	75.91 a
AH 6K	7.46 a	837.6 a	97.14 a	279.63 a	76.28 a
5018 S	6.68 a	755.9 a	96.09 a	260.27 a	75.35 a
CB 756K	5.37 a	641.4 a	89.09 a	250.86 a	75.19 a
AH 10bK	7.64 a	527.0 a	89.58 a	246.07 a	75.69 a
Uninoculated	4.63 a	511.3 a	88.31 a	239.45 a	76.05 a
SE	1.72	133.8	6.06	14.89	2.82

*Means followed by a common letter are not significantly different at the 5% level of probability as determined by Duncan's Multiple Range Test

leaf %N, leghaemoglobin content in nodules and nitrogenase activity (Schiffman & Lobel, 1970; Koch & Evans, 1966). Accurate measurement of nitrogen fixation is essential for correct selection of *Rhizobium* strains. For groundnuts, %N or dry weight of the shoot seems to offer the best assessment for plant benefit from the symbiosis.

Lack of response to inoculation may be due to adequate nodulation by indigenous rhizobia, unfavourable conditions for survival of introduced *Rhizobium* strains or failure of inoculant strains to compete for nodule sites with native strains. Natural nodulation was good as determined by the number of nodules on roots of plants in uninoculated plots. Application of high rates of inoculum is required to overcome the effects of the indigenous *Rhizobium* population (Kapusta & Rouwenhorst, 1973; Weaver & Frederick, 1974). This is provided that inoculant strains are competitive. In this instance, it would seem that the inocula were not sufficiently concentrated, nor were inoculant strains highly competitive.

Recovery of kanamycin-resistant strains from uninoculated plots indicates either the presence of naturally resistant strains in the soil or contamination of uninoculated plots from nearby inoculated plots. The case of the former is rare (Obaton, 1971) but in the event of its occurrence, results obtained using antibiotic resistance as an ecological marker, must be interpreted with caution.

Groundnut yields obtained here are relatively low; the world average yield is 884 kg ha^{-1} (Dart & Krantz, 1976). This was mainly due to an excessively wet period just prior to harvesting which caused mature pods to sprout, and retarded pod set owing to waterlogging. Nevertheless, the increases in yield of inoculated plots over uninoculated plots suggest that given correct conditions a significant response to inoculation is possible.

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