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The response of groundnut to inoculation at field sites in Peninsular Malaysia

(Gerak balas kacang tanah terhadap penginokulatan di tapak percubaan di Semenanjung Malaysia)

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Key words: *Arachis hypogaea*, bradyrhizobia, inoculation, Malaysia, nodulation

Abstrak

Pokok kacang tanah telah ditanam dalam enam tapak percubaan di Semenanjung Malaysia bagi menilai keperluan untuk diinokulat dengan bradyrhizobia. Strain-strain bradyrhizobia yang dapat membentuk bintil pada akar pokok kekacang telah ditemui pada keenam-enam tapak percubaan. Penginokulatan dengan strain-strain MS13 dan NC92 yang diketahui sangat berkesan didapati gagal untuk meningkatkan bilangan bintil tetapi dapat meningkatkan berat bintil pada dua tapak percubaan. Berat purata bintil pada tiap-tiap tapak percubaan tidak dipengaruhi oleh perlakuan. Kedua-dua strain NC92 dan MS13 dapat membentuk lebih banyak bintil pada satu tapak yang sama tetapi pada tapak yang lain pula hanya strain NC92 yang berkesan. Keadaan ini menunjukkan ketentuan di antara strain dan tapak percubaan. Penginokulatan dan penambahan N tidak meningkatkan pertumbuhan tampang pada hari ke-50 atau hasil biji kacang; kedua-dua pembolehubah nampaknya tidak berkaitan.

Abstract

Groundnuts were sown at six sites in Peninsular Malaysia to assess the need for inoculation with bradyrhizobia. Strains of bradyrhizobia capable of nodulating groundnuts occurred at all the six sites. Inoculation with the highly effective strains MS13 and NC92 failed to increase nodule number but it increased nodule mass at two sites. Mean nodule mass at each site was unaffected by treatment. More nodules were formed by both the strains NC92 and MS13 at one site but only by NC92 at another, indicating strain and site specificity. Neither inoculation nor the addition of N increased vegetative growth at 50 days or seed yield; these two variables appeared to be unrelated.

Introduction

Arachis hypogaea (groundnut) is the most important grain legume in Peninsular Malaysia where 60% of production originates from riverine areas. Groundnut is also grown as an intercrop in young rubber or oil palm plantations but the expected area for expansion is likely to be in rotation with single crop, rainfed rice. In most commercial planting groundnut is

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not inoculated.

Lack of nitrogen has been proposed by Foster (1981) as one explanation for the poor yields (300–1 600 kg/ha) of groundnut in Malaysia (Au Yong 1966). Groundnuts nodulate readily in cultivated areas with a soil pH of 4.5 (H_2O), but in a pot trial with peat soil, nodulation occurred only at pH values above 4.7 and maximum N_2 fixation occurred at pH 6.5 (Sundram 1979). In acid soils, nodulation is adversely affected when the aluminium saturation exceeds 75% (Foster 1981). The effectiveness of nodulation and nitrogen fixation of the naturalised bradyrhizobia with groundnut in Malaysia is unknown.

In this paper the authors assess the need to inoculate groundnut in five agro-ecological zones of Peninsular Malaysia as a preliminary step to studies aimed at selecting strains of bradyrhizobia adapted to these environments.

Materials and methods

Field sites

Six sites with different agro-ecological environments were chosen. The following list of sites gives their names, map references, soil types and previous history of cultivation. All pH values were determined on a 1:2.5 soil: water suspension except for Pontian peat-soil where 1:5 suspension was used.

Site 1: Serdang 1: 3°05'N 101°40'E, Ultisol, pH 4.8, previously cropped with cassava following rubber undersown with *Calopogonium, Centrosema* and *Pueraria.*

Site 2: Serdang 2: 3°05'N 101°40'E, Inceptisol, pH 4.5, previously planted with sweet potatoes following rubber undersown with *Calopogonium, Centrosema and Pueraria.*

Site 3: Bertam: 5°30'N 100°30'E, Inceptisol, pH 4.6, recently cleared of rubber and previously sown with *Calopogonium, Centrosema* and *Pueraria.*

Site 4: Padang Serai: 5°27'N 100°00'E, Inceptisol, pH 5.1, previously sown with *Calopogonium, Centrosema* and *Pueraria.*

Site 5: Jeram Pasu: 5°41'N 102°23'E, Entisol, pH 4.9, recently cleared of rubber.

Site 6: Pontian: 1°26'N 103°20'E, Histosol, pH 3.7, previously under secondary forest.

At each site, land was prepared by ploughing, harrowing and rotovation. Lime, as ground magnesium limestone (approximately 34% CaO and 19% MgO), was applied 2 weeks before planting at 1 t/ha except at Pontian where 4 t/ha was applied.

Experimental procedures

Spanish type groundnuts (cv. Matjam) were sown in 5 m x 5 m plots with plants spaced 10 cm apart within rows and 50 cm between rows. The plots were separated by drains (0.5 m wide and 0.3 m deep) to reduce the spread of introduced bradyrhizobia. Plots were arranged in randomised complete block design with four replicates of the following four treatments: uninoculated, uninoculated plus 100 kg N/ha as urea applied in two equal dressings at planting and at 50% flowering, inoculated at sowing with *Bradyrhizobium* strain NC92 (obtained from Dr. G. H. Elkan, NCSU Raleigh, USA) or MS13 (isolated from nodule of *Gliricidia* sp., Serdang) prepared in sterilised peat providing approximately 2.5 x 10^6 bradyrhizobia per seed.

After planting, the following fertilizers were broadcast by hand at the rate of 12 kg P/ha as triple superphosphate, 14 kg K/ha as potassium chloride and 2.22 kg Mo/ha as ammonium molybdate. At Pontian, in addition to basal fertilizers all plots received $CuSO_4$ (2 kg/ha), Borax (15 kg/ha), $ZnSO_4$ (5 kg/ha), and $MnSO_4$ (15 kg/ha).

At Serdang 1, Serdang 2 and Jeram Pasu, plants were sprinkler irrigated as required during the first month; at Bertam they were hand-watered until rainfall was regular. At Padang Serai and Pontian, plots were entirely rainfed. All plots were hand-weeded and sprayed with dicofol *(Kelthane)* and *Malathion* at recommended rates. At approximately 7 weeks (50% flowering), 10 plants were dug at random from a 1-m strip across the central eight rows of each plot and the following determinations were made: fresh and dry weight of shoots, nodule number and nodule dry weight per plant and total plant nitrogen (by Kjeldahl digestion). The roots of a further five plants/plot were sampled at random to estimate the proportion of nodules formed by the two introduced strains. These were identified by fluorescein isothiocyanate (FITC) labelled antibody applied to nodule squashes. Seed yield at maturity (100–110 days) was measured by harvesting all the plants from within a 2.5–m strip across the central eight rows of each plot.

Results

Native strains of bradyrhizobia occurred at all sites as evidenced by nodulation in the uninoculated treatments *(Table 1)*. Inoculations with either MS13 or NC92 failed to significantly ($p < 0.05$) increase the number of nodules at any site (data not presented) but inoculation with NC92 increased nodule mass at Pontian and Jeram Pasu and MS13 at Pontian only.

The number of nodules/plant was least at Pontian where nodule mass/plant was greatest (102 mg) *(Table 1)*. Nodule numbers were highest at the Serdang and Bertam sites where nodule mass/plant was very low (32–48 mg). Strain NC92 consistently formed more nodule mass/plant than MS13; this difference was significant when pooled over sites. Mean nodule mass was also directly related to site *(Table 2)*. Nodule size at each site was remarkably similar irrespective of treatment but varied between sites by up to 13-fold. Inoculation with NC92 increased the proportion of nodules formed by this serological group compared with uninoculated plots at Serdang 1, Jeram Pasu and Pontian and inoculation with MS13 at only Jeram Pasu and Padang Serai *(Table 3)*. A high proportion of the naturalised rhizobia cross-reacted with sera of both inoculant strains; this was particularly so with MS13.

Table 1. Nodule number and the effect of inoculation on nodule mass at six sites

Site	Nodules/plant*	Nodule mass** (mg/plant)				
		MS13	NC92	+N	−N	Site mean
Bertam	116	28	38	36	28	32
Jeram Pasu	87	83	108	56	73	80
Padang Serai	89	79	97	55	79	78
Pontian	29	100	188	53	68	102
Serdang 1	146	53	53	30	55	48
Serdang 2	170	38	44	42	58	45
Treatment mean		63	88	45	60	

LSD ($p = 0.05$) Nodule number = 30
LSD ($p = 0.05$) Nodule mass:
Site means = 16
Treatment means = 13
Site x treatment = 32
*Each value is the mean of four replicates each of 40 plants
**Each value is the mean of four replicates each of 10 plants

Table 2. Mean nodule mass (mg) at six sites

Site	Treatment				Site mean
	MS13	NC92	+N	−N	
Bertam	0.27	0.33	0.28	0.30	0.29
Jeram Pasu	1.02	0.99	0.88	0.90	0.93
Padang Serai	0.93	0.86	0.69	1.03	0.87
Pontian	4.48	3.62	3.42	4.02	3.89
Serdang 1	0.33	0.35	0.29	0.34	0.33
Serdang 2	0.31	0.29	0.27	0.29	0.29
Treatment mean	1.23	1.07	0.95	1.15	

LSD ($p = 0.05$); Site mean = 0.28
Treatment means not significantly different ($p < 0.05$)

Table 3. The percentage of nodules formed by strains which reacted with antiserum of the inoculant strains

Site and treatment	% nodules reacting with antisera to	
	NC92	MS13
Serdang 1		
NC92	40	15
MS13	19	36
+N	17	45
−N	37	57
Serdang 2		
NC92	40	27
MS13	36	58
+N	40	59
−N	18	40
Bertam		
NC92	14	29
MS13	6	26
+N	26	31
−N	5	35
Jeram Pasu		
NC92	20	37
MS13	8	65
+N	7	40
−N	8	30
Padang Serai		
NC92	29	6
MS13	37	69
+N	37	35
−N	11	39
Pontian		
NC92	68	42
MS13	13	42
+N	29	14
−N	27	49

Neither inoculation nor the addition of N increased shoot dry weight or total N in the shoot or grain yield at any one site (data not shown) but all these parameters varied considerably between sites *(Table 4)*. There was no apparent relationship between grain yield and shoot growth or nitrogen content at 50 days after sowing. For example, at Bertam, where shoot dry weight and total N were highest, grain yield was not significantly greater than the lowest yield at Serdang 2.

Discussion

These trials were established at six sites where groundnuts had not previously been grown to assess whether inoculation with selected strains of *Bradyrhizobium* was beneficial. Inoculation did not significantly increase yield at any site, however, neither did the application of 100 kg N per hectare. With the exception of the trial at Pontian, a highly acid peat with likely mineral imbalances, nodule number was high. It is likely that environmental factors limited pod yield and hence the response to inoculation is best assessed by considering the nodulation data. Further evidence to support this is the lack of a relationship between final grain yield and shoot dry weight or nitrogen at 50 days.

Consideration of nodule number in association with nodule dry weight indicated that the inverse relationship established for clover (Nutman 1967)

Table 4. Shoot dry weight and nitrogen content at 50 days and grain yield at maturity at six sites

	Mean shoot dry wt. (g/plant)	Shoot N (mg/plant)	Grain yield (kg/ha)
Bertam	19.0	630	1 386
Jeram Pasu	13.8	360	1 510
Padang Serai	12.3	440	3 762
Pontian	9.0	180	1 588
Serdang 1	6.9	260	2 328
Serdang 2	12.2	370	1 081
LSD (p = 0.05)	2.1	89	349.5

Each value is the mean of four replicates each of 10 plants

applied to groundnut in these experiments. The results clearly indicate nodule size was determined by conditions prevailing at each site rather than by bacterial strain or the addition of nitrogen. The overall consistency of nodule size at each site is particularly striking.

Inoculation significantly increased nodule mass at only two sites, Jeram Pasu and Pontian and there was a large increase at Padang Serai although this was not significant (p <0.05) when compared with the -N treatment. Serological typing of isolates from nodules indicated a general failure to establish a high proportion formed by NC92 or MS13. Only at the above three sites did inoculation with one or other strain increase nodulation of their sera-group by more than 30%. Increasing nodulation by these elite strains did not increase vegetative growth at 50 days or grain yield suggesting that the naturalised bradyrhizobia in these soils were equally effective with groundnut under the prevailing conditions. Strain NC92 produced consistently more nodule mass/ plant than MS13, this difference was significant at Pontian and when averaged over all sites. It was surprising that the highest nodule mass formed at Pontian occurred when at that time, plant growth was poor (shoot dry weight 9 g compared with 19 g at Bertam). As would be expected the application of nitrogen decreased nodulation (nodule mass/plant) at five of the six sites but this difference failed to reach significance in these trials.

Although there were no yield responses to inoculation at these sites, such responses are not uncommon in Peninsular Malaysia (Faizah Abd Wahab, RRIM, pers. comm. 1987). Over the sites studied, strains NC92 and MS13 were not superior than the naturalised bradyrhizobia. There appears to be no necessity to inoculate with bradyrhizobia at least for areas previously cropped with legumes until a more superior strain is found. Opportunity was taken to isolate strains, likely to be both effective and competitive in these soils, from nodules formed in uninoculated plots. These will be evaluated in the next phase of the programme.

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