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Optimization of Rhizobium Inoculation and Phosphorus Nutrition for Enhanced Nodulation, Nutrient-Use Efficiency and Productivity of Groundnut under Semi-arid Conditions

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ABSTRACT

Background: Groundnut (*Arachis hypogaea* L.) productivity in semi-arid India is constrained by poor nodulation and low phosphorus (P) availability. How a locally adapted rhizobial strain interacts with graded P supply has not been quantified for the red soils of Telangana.

Methods: A *kharif* field experiment (randomized block design, 3 × 4 factorial, three replications) was conducted at SR University, Warangal, with groundnut cv. Dharani (K-9). Treatments were three Rhizobium regimes (uninoculated; recommended commercial *Bradyrhizobium* culture; locally isolated phosphate-solubilizing native strain) and four P levels (0, 20, 40 and 60 kg P_2O_5 ha^{-1}). Nodulation, growth, nutrient uptake, yield, quality and economics were recorded.

Result: Rhizobium, P level and their interaction significantly (Pd"0.05) affected nodulation, N and P uptake, pod and haulm yield and net return. The local strain with 60 kg P_2O_5 ha^{-1} gave the highest nodule number (80.5 $plant^{-1}$), nodule dry weight (149.8 mg $plant^{-1}$), N uptake (138.0 kg ha^{-1}), P uptake (10.9 kg ha^{-1}), pod yield (4370 kg ha^{-1}) and net return (Rs 202,422 ha^{-1}; benefit: cost ratio 4.62), which were 86.7% and 129.4% above the uninoculated, unfertilized control for pod yield and net return, respectively. The local strain out-yielded the recommended culture at every P level (by 9.5-11.2%) and with 40 kg P_2O_5 ha^{-1} it matched the recommended culture at 60 kg. Agronomic efficiency of applied P was 23.6-24.1 kg pods kg^{-1} P_2O_5 with the local strain against 15.5-16.2 without inoculation and did not decline up to 60 kg P_2O_5 ha^{-1}. Combining a locally adapted Rhizobium with 40-60 kg P_2O_5 ha^{-1} is a low-cost strategy for profitable rainfed groundnut.

Key words: *Arachis hypogaea*, Biological nitrogen fixation, Nodulation, Phosphorus use efficiency, Rhizobium inoculation, Semi-arid agriculture.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is the most important annual oilseed legume of the semi-arid tropics, valued for edible oil, protein-rich kernels and nitrogen-enriching residues. India is the second largest producer, but most of the area lies in rainfed, resource-poor tracts where shallow, light-textured soils, erratic rainfall and low native fertility constrain productivity (Directorate of Economics and Statistics, 2024). As a nodulating legume, groundnut obtains much of its nitrogen (N) through symbiotic fixation, yet this is often limited by scarce or poorly competitive native rhizobia and by phosphorus (P) deficiency, because P is indispensable for nodule initiation, bacteroid energy metabolism and nitrogenase activity (Sulieman and Tran, 2015).

P is required for nodule primordium formation, for the high respiratory and phosphorylation demand of bacteroids and for nodule acid phosphatases that mobilize internal P reserves when supply is limiting (Zhong *et al*., 2023). Legumes therefore have a high P requirement, aggravated in semi-arid calcareous and red soils where P is fixed as calcium or iron/aluminium phosphates (Chen *et al*., 2023; Zhong *et al*., 2023). Combined application of rhizobial inoculant and P fertilizer often gives synergistic gains in nodulation and yield, because adequate P amplifies the response to inoculation while active nodulation improves P acquisition (Asante *et al*., 2020; Míguez-Montero *et al*., 2020; Naveen and Senthilkumar, 2021).

Strain adaptation is a further, less-examined dimension. Commercial cultures do not always out-compete indigenous populations or tolerate local stresses as well as isolates from the same niche, so locally adapted strains may give better nodule occupancy, persistence and symbiotic effectiveness (Gouda *et al*., 2018; Mendoza-

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Suárez *et al.*, 2021). Effective native groundnut rhizobia have been identified in Ghana (Osei *et al.*, 2018) and native isolates of groundnut have also been characterized and evaluated (Chandrakanta *et al.*, 2024). However, studies on Rhizobium-P interactions come largely from Africa (Asante *et al.*, 2020; Kyei-Boahen *et al.*, 2017) or have used consortium or phosphate-solubilizing cultures rather than a locally isolated *Bradyrhizobium* strain (Bajya and Sharma, 2024; Ravi *et al.*, 2024) and quantitative data on how a locally adapted strain performs against a recommended culture across a graded P series and how this translates into nutrient-use efficiency and profitability, are meagre for the semi-arid red soils of Telangana.

This study therefore aimed at evaluating (i) the effect of *Rhizobium* inoculation on growth, nodulation and nutrient uptake of groundnut, (ii) the optimum P level, (iii) the *Rhizobium* × P interaction and (iv) the economics of the resulting combinations, in order to recommend a practical inoculant and P package for rainfed groundnut.

MATERIALS AND METHODS

Site and soil A field experiment was conducted during the *Kharif* (rainy) season of 2025 at the Instructional and Research Farm, School of Agriculture, SR University, Warangal, Telangana, India (18.0°N, 79.6°E; 265 m above mean sea level). The climate is semi-arid tropical, with a hot, dry summer and monsoon rains (June-October) supplying most of the 950-1000 mm mean annual rainfall; soils are shallow to medium-deep red sandy loams (Alfisols). The experimental soil (0-15 cm) was sandy loam, neutral (pH 7.2, 1:2.5), non-saline (EC 0.28 dS m^{-1}), low in organic carbon (0.42%, Walkley-Black) and available N (188 kg ha^{-1}, alkaline permanganate) and medium in available P (16.4 kg P_2O_5 ha^{-1}, Olsen) and K (218 kg K_2O ha^{-1}, neutral normal ammonium acetate).

Treatments and crop management

The experiment followed a randomized block design with a 3 × 4 factorial arrangement and three replications (36 plots; gross plot 4.2 × 3.6 m). Factor A comprised three Rhizobium regimes: R1, uninoculated control; R2, seed treatment with a recommended commercial *Bradyrhizobium* sp. (*Arachis*) culture (200 g per 10 kg seed, ≥10^8 viable cells g^{-1}) in jaggery slurry; and R3, seed treatment at the same rate with a locally isolated, phosphate-solubilizing native *Bradyrhizobium* strain multiplied in the host institution's microbiology laboratory. Factor B comprised four P levels (P0, P20, P40 and P60 = 0, 20, 40 and 60 kg P_2O_5 ha^{-1}) applied as single super phosphate at sowing. All plots received a basal 20 kg N and 30 kg K_2O ha^{-1}. Groundnut cv. Dharani (K-9), a bunch-type, semi-spreading, 105-110-day cultivar recommended for rainfed *kharif* cultivation in Telangana and Andhra Pradesh, was sown at 30 cm × 10 cm. Gap filling, weeding, plant protection and life-saving irrigation followed the recommended package of practices and were uniform across treatments.

Observations

Five randomly tagged plants per plot were used to record plant height, leaf area index (LAI; non-destructive leaf-area-ratio method), dry matter and SPAD chlorophyll readings (SPAD-502, Konica Minolta) at 60 days after sowing (DAS), when nodulation differences are most discernible. Roots were excavated and washed; nodules were counted and oven-dried at 70±2°C and root dry weight was recorded. Tissue N and P were estimated by the micro-Kjeldahl and vanado-molybdo-phosphoric yellow colour methods and multiplied by dry matter to obtain uptake (kg ha^{-1}). At harvest, pods $plant^{-1}$, pod weight $plant^{-1}$, shelling percentage and 100-kernel weight were recorded; pod and haulm yields were recorded plot-wise and expressed at 8% pod moisture. Kernel oil was determined by Soxhlet extraction and protein as kernel N × 6.25.

Efficiency and economics

Within each Rhizobium regime, agronomic efficiency of applied P (AE, kg pods kg^{-1} P_2O_5) was calculated as (pod yield at a given P level - pod yield at P0)/P_2O_5 applied and additional P and N uptake per kg P_2O_5 were derived likewise from uptake means. Cost of cultivation was worked out treatment-wise from prevailing input costs (seed, inoculant, fertilizer, labour) and gross return from prevailing prices of pods and haulm. Net return was gross return minus cost of cultivation and benefit:cost ratio (BCR) was gross return divided by cost of cultivation.

Statistical analysis

Data were subjected to analysis of variance for a two-factor factorial in randomized block design (Gomez and Gomez, 1984), with replication, Rhizobium, P level and their interaction as sources of variation. Means were compared using the critical difference (CD) at P = 0.05 from the pooled error mean square; standard errors of the mean (SEm±) are also reported. Computations were done in Python 3.11 (pandas, statsmodels).

RESULTS AND DISCUSSION

Growth and physiological attributes

Rhizobium inoculation and P level significantly influenced all growth attributes at 60 DAS (Table 1). Plant height, LAI, dry matter and SPAD index were highest with the locally adapted strain (52.02 cm, 3.38, 51.08 g $plant^{-1}$ and 43.13), followed by the recommended culture (48.78 cm, 3.15, 47.48 g and 41.03) and the uninoculated control (44.19 cm, 2.81, 43.03 g and 39.01). Each P increment further improved growth; 60 kg P_2O_5 ha^{-1} increased plant height by 21.9% and dry matter by 35.4% over no P. The R × P interaction was significant only for plant height and SPAD index, so LAI and dry matter responded additively. Higher SPAD readings indicate better leaf N status, consistent with greater N fixation and uptake (Zhong *et al.*, 2023).

Nodulation and root growth

Nodulation was the most responsive trait (Table 1). Relative to uninoculated plants (40.7 nodules and 77.7 mg nodule dry weight plant^{-1}), the recommended culture increased nodule number by 32.5% and nodule dry weight by 30.1% and the local strain by 56.0% and 54.2%, respectively. Nodule number and dry weight rose by 80.8% and 70.3% from 0 to 60 kg P_2O_5 ha^{-1}. The interaction was significant (Table 3): the nodule advantage of the local strain over uninoculated plants widened from 17.9 plant^{-1} at P0 to 27.3 at P60 and the maximum (80.5 nodules and 149.8 mg plant^{-1}) occurred with R3P60. Even without added P, the local strain (46.1 nodules) out-nodulated uninoculated plants given 20 kg P_2O_5 ha^{-1} (37.2). Root dry weight followed the same trend (Table 1).

This synergy is well documented: P is required for nodule development and nitrogenase function and P starvation impairs nodule development and N fixation (Wang *et al*., 2020; Zhong *et al*., 2023). Positive inoculant × P interactions on nodulation have likewise been reported in groundnut (Asante *et al*., 2020) and cowpea (Kyei-Boahen *et al*., 2017), as has greater nodulation with P plus a microbial culture in groundnut (Ravi *et al*., 2024) and with combined N and P in bambara groundnut (Hasan *et al*., 2021). The local strain out-nodulated the commercial culture at every P level (by 7.5-10.8 nodules plant^{-1}), probably reflecting better compatibility with the host and local soil and its phosphate-solubilizing ability; competitiveness and persistence of inoculant strains are decisive for nodule occupancy (Mendoza-Suárez *et al*., 2021). Strain occupancy was not, however, assessed here.

Nutrient uptake

N and P uptake increased significantly with rhizobium and P level (Table 1). Mean N uptake was 24.1% (recommended) and 41.0% (local strain) higher than in uninoculated plants and P60 raised it by 43.5% over P0; corresponding P uptake gains were 9.9%, 15.9% and 38.7%. Maximum N (138.0 kg ha^{-1}) and P uptake (10.9 kg ha^{-1}) occurred with R3P60 (Table 3), 101.7% and 57.3% above the control (R1P0). Greater N uptake by inoculated plants reflects enhanced N fixation, while the larger root systems (Table 1) and the phosphate-solubilizing strain (Bajya and Sharma, 2024) probably improved P acquisition, for which root growth is central in legumes (Chen *et al*., 2023).

Yield attributes and yield

Pods plant^{-1}, pod weight, shelling percentage and 100-kernel weight all increased with inoculation and P (Table 2); the interaction was significant only for pods plant^{-1} and pod weight and R3P60 produced the most pods (43.1 plant^{-1}; Table 3). Mean pod yield was 2818, 3313 and 3666 kg ha^{-1} for R1, R2 and R3 and 2665, 3063, 3457 and 3877 kg ha^{-1} at 0, 20, 40 and 60 kg P_2O_5 ha^{-1}; haulm yield followed a similar trend (Table 2). The interaction was significant (Table 3): R3P60 recorded the highest pod yield (4370 kg ha^{-1}) and haulm yield (4492 kg ha^{-1}), 86.7% and 56.3% above the

Table 1: Growth, nodulation and nutrient uptake of groundnut as influenced by Rhizobium inoculation (R) and phosphorus (P) levels (main effects).

Treatment	Plant height (cm)	LAI	Dry matter (g plant^{-1})	SPAD index	Nodules plant^{-1} (no.)	Nodule dry wt. (mg plant^{-1})	Root dry wt. (g plant^{-1})	N uptake (kg ha^{-1})	P uptake (kg ha^{-1})
R1: Uninoculated	44.19	2.81	43.03	39.01	40.73	77.73	3.80	82.67	8.09
R2: Recommended culture	48.78	3.15	47.48	41.03	53.95	101.10	4.27	102.56	8.89
R3: Locally adapted strain	52.02	3.38	51.08	43.13	63.54	119.86	4.69	116.57	9.38
P0	43.61	2.72	40.01	37.98	37.63	73.58	3.66	82.36	7.33
P20	46.72	3.01	45.01	40.00	47.88	91.52	4.05	95.02	8.41
P40	49.81	3.24	49.60	42.18	57.41	107.87	4.43	106.83	9.24
P60	53.17	3.49	54.17	44.08	68.04	125.29	4.87	118.18	10.17
SEm± (R)	0.176	0.013	0.245	0.067	0.328	0.519	0.023	0.383	0.045
CD (R)	0.517	0.037	0.719	0.197	0.962	1.523	0.069	1.124	0.132
SEm± (P)	0.204	0.015	0.283	0.077	0.379	0.600	0.027	0.442	0.052
CD (P)	0.597	0.043	0.830	0.227	1.111	1.759	0.079	1.298	0.153
SEm± (R × P)	0.353	0.026	0.490	0.134	0.656	1.039	0.047	0.766	0.090
CD (R × P)	1.034	NS	NS	0.393	1.925	3.046	NS	2.248	0.264

R1: Uninoculated control; R2: Recommended commercial *Bradyrhizobium* culture; R3: Locally adapted native strain; P0-P60: 0-60 kg P_2O_5 ha^{-1}; LAI: Leaf area index; SPAD: Chlorophyll meter reading; SEm±: Standard error of mean; CD: Critical difference at 5% probability; NS: Non-significant.

Table 2: Yield attributes, yield, kernel quality and economics of groundnut as influenced by rhizobium inoculation (R) and phosphorus (P) levels (main effects).

Treatment	Pods plant^{-1} (no.)	Pod weight (g plant^{-1})	Shelling (%)	100-kernel wt. (g)	Pod yield (kg ha^{-1})	Haulm yield (kg ha^{-1})	Oil (%)	Protein (%)	Net return (Rs ha^{-1})	BCR
R1: Uninoculated	24.07	22.55	66.01	40.62	2817.75	3242.50	47.13	23.92	114598	3.14
R2: Recommended culture	29.99	26.02	67.71	42.12	3312.83	3629.25	47.62	24.42	142473	3.62
R3: Locally adapted strain	34.36	28.71	68.98	43.35	3665.75	3924.17	48.08	24.90	162760	3.97
P0	22.26	21.58	65.40	39.67	2664.89	3135.22	47.01	23.97	106402	3.02
P20	26.92	24.46	66.99	41.02	3063.33	3457.67	47.44	24.32	128716	3.40
P40	31.70	27.14	68.34	42.89	3456.67	3749.67	47.81	24.58	150608	3.76
P60	37.01	29.86	69.53	44.56	3876.89	4052.00	48.19	24.80	174046	4.14
SEm± (R)	0.141	0.078	0.105	0.147	15.265	10.614	0.035	0.039	848.756	0.015
CD (R)	0.414	0.228	0.309	0.432	44.772	31.130	0.102	0.113	2489.315	0.045
SEm± (P)	0.163	0.090	0.121	0.170	17.627	12.256	0.040	0.045	980.059	0.018
CD (P)	0.478	0.264	0.356	0.499	51.698	35.946	0.118	0.131	2874.413	0.052
SEm± (R × P)	0.282	0.156	0.210	0.295	30.531	21.228	0.070	0.077	1697.511	0.031
CD (R × P)	0.828	0.457	NS	NS	89.544	62.260	NS	NS	4978.630	0.090

R1: Uninoculated control; R2: Recommended commercial *Bradyrhizobium* culture; R3: Locally adapted native strain; P0-P60: 0-60 kg P_2O_5 ha^{-1}; LAI: Leaf area index; SPAD: Chlorophyll meter reading; SEm±: Standard error of mean; CD: Critical difference at 5% probability; NS: Non-significant. BCR: Benefit:cost ratio; Rs: Indian rupees.

Table 3: Interaction of rhizobium inoculation (R) and phosphorus (P) level on nodulation, nutrient uptake, yield and economics of groundnut.

Treatment	Nodules plant^{-1} (no.)	Nodule dry wt. (mg plant^{-1})	N uptake (kg ha^{-1})	P uptake (kg ha^{-1})	Pods plant^{-1} (no.)	Pod yield (kg ha^{-1})	Haulm yield (kg ha^{-1})	Net return (Rs ha^{-1})	BCR
R1P0	28.20	56.37	68.43	6.93	18.23	2341.00	2873.33	88232	2.70
R1P20	37.17	70.40	77.87	7.73	21.83	2656.33	3162.00	105857	3.00
R1P40	44.33	84.03	87.23	8.50	25.93	2961.33	3343.67	122446	3.28
R1P60	53.23	100.13	97.13	9.20	30.27	3312.33	3591.00	141855	3.59
R2P0	38.60	74.63	83.27	7.37	22.67	2698.67	3176.33	108242	3.05
R2P20	48.27	93.47	97.40	8.53	27.53	3096.33	3475.67	130397	3.42
R2P40	58.53	110.33	110.17	9.27	32.10	3508.00	3791.67	153390	3.81
R2P60	70.40	125.97	119.40	10.40	37.67	3948.33	4073.33	177861	4.20
R3P0	46.10	89.73	95.37	7.70	25.87	2955.00	3356.00	122733	3.31
R3P20	58.20	110.70	109.80	8.97	31.40	3437.33	3735.33	149895	3.77
R3P40	69.37	129.23	123.10	9.97	37.07	3900.67	4113.67	175988	4.20
R3P60	80.50	149.77	138.00	10.90	43.10	4370.00	4491.67	202422	4.62
SEm± (R × P)	0.656	1.039	0.766	0.090	0.282	30.531	21.228	1697.511	0.031
CD (R × P)	1.925	3.046	2.248	0.264	0.828	89.544	62.260	4978.630	0.090

R1P0 denotes R1 with P0 and so on; R1: Uninoculated control; R2: Recommended commercial *Bradyrhizobium* culture; R3: Locally adapted native strain; P0-P60: 0-60 kg P_2O_5 ha^{-1}; LAI: Leaf area index; SPAD: Chlorophyll meter reading; SEm±: Standard error of mean; CD: Critical difference at 5% probability; NS: Non-significant.

uninoculated, unfertilized control. The local strain out-yielded the recommended culture at every P level (by 256-422 kg ha^{-1}, or 9.5-11.2%; CD = 89.5) and R3P40 (3901 kg ha^{-1}) was at par with R2P60 (3948 kg ha^{-1}), so the local strain saved 20 kg P_2O_5 ha^{-1}. Even R3P20 (3437 kg ha^{-1}) exceeded uninoculated plants given 60 kg P_2O_5 ha^{-1} (3312 kg ha^{-1}). Comparable gains from combined inoculation and P have been reported in groundnut and cowpea (Asante *et al.*, 2020; Kyei-Boahen *et al.*, 2017; Naveen and Senthilkumar, 2021; Ravi *et al.*, 2024) and here reflect more nodules, greater N and P uptake and better pod set.

Nutrient-use efficiency

AE was 15.5-16.2 kg pods kg^{-1} P_2O_5 without inoculation, 19.9-20.8 with the recommended culture and 23.6-24.1 with the local strain and did not decline with P dose; inoculation thus raised AE by 26-30% (R2) and 46-53% (R3). N uptake per kg P_2O_5 was likewise stable with the local strain (0.69-0.72 kg). In contrast, additional P uptake per kg P_2O_5 fell from 0.064 kg at P20 to 0.057 and 0.053 kg at P40 and P60 with the local strain, indicating diminishing recovery of applied P, as expected in soils that fix P (Lambers, 2022). Thus, although P recovery declined at higher doses, yield efficiency did not and the response had not plateaued at 60 kg P_2O_5 ha^{-1}.

Kernel quality

Kernel oil and protein rose significantly but modestly with inoculation (oil 47.13-48.08%, protein 23.92-24.90% from R1 to R3) and P (oil 47.01-48.19%, protein 23.97-24.80% from P0 to P60), without interaction (Table 2), consistent with improved N and P nutrition.

Economics

Net return and BCR increased with both factors (Table 2). Relative to uninoculated plants, inoculation raised mean net return by Rs 27,875 (recommended) and Rs 48,162 ha^{-1} (local strain) while adding only Rs 900-1,200 ha^{-1} to the cost of cultivation and 60 kg P_2O_5 ha^{-1} added Rs 67,644 over no P. R3P60 gave the highest net return (Rs 202,422 ha^{-1}) and BCR (4.62), 129.4% above the control (Rs 88,232) and R3P40 (Rs 175,988; BCR 4.20) was at par with R2P60 (Rs 177,861; BCR 4.20). Each 20 kg P_2O_5 ha^{-1} increment added Rs 26,093-27,162 ha^{-1} with the local strain, so 60 kg P_2O_5 ha^{-1} remained the most profitable dose tested, in agreement with Ravi *et al.* (2024), who recorded the highest net return and benefit:cost ratio in groundnut with 60 kg P ha^{-1} plus a microbial culture.

Conclusion

Rhizobium inoculation and P nutrition acted synergistically on nodulation, nutrient uptake and productivity of rainfed groundnut on a semi-arid red sandy loam. The locally isolated phosphate-solubilizing *Bradyrhizobium* strain outperformed both the uninoculated control and the recommended commercial culture at all P levels and with 40 kg P_2O_5 ha^{-1} it matched the commercial culture at 60 kg P_2O_5 ha^{-1}. Nodulation, nutrient uptake, pod and haulm yield and net return rose with each P increment up to 60 kg P_2O_5 ha^{-1} without any decline in agronomic efficiency, so the local strain with 60 kg P_2O_5 ha^{-1} gave the highest pod yield (4370 kg ha^{-1}) and net return (Rs 202,422 ha^{-1}; BCR 4.62), 86.7% and 129.4% above the control. Where P supply is limited, the local strain with 40 kg P_2O_5 ha^{-1} is an economical alternative. Because the study covered one season and site and response had not plateaued at 60 kg P_2O_5 ha^{-1}, multi-location, multi-season validation with higher P rates and nodule occupancy assessment is needed.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

This study was a field crop trial and did not involve human participants, human data or animals; ethics approval and informed consent were therefore not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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