

GROWTH AND NODULATION IMPROVEMENT OF PEANUT BY RHIZOBIAL AND CYANOBACTERIAL APPLICATIONS.

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ABSTRACT

A pot experiment was conducted in the greenhouse of the Agricultural Research Center (ARC) to evaluate the response of two cultivars of peanut to rhizobial inoculation and cyanobacterial application. The results indicated that the biomass of peanut plants, significantly increased due to rhizobia inoculation with strains Kh8 and Kh11. Soil treatment with some cyanobacterial filtrates enhanced growth and nodulation of the legume plant. Plants of peanut cv. Georgia exhibited higher dry weights of both roots ($1.93 \text{ mg plant}^{-1}$) and shoots ($6.43 \text{ mg plant}^{-1}$), as well as better nodulation parameters ($241 \text{ nodules plant}^{-1}$ of 0.51 mg). In addition, the data revealed that N-content of peanut for both tested cultivars increased as a result of mixed rhizobial inoculation with Kh8 and Kh11 and cyanobacterial treatments. In this respect, 146 mg N/plant and 99 mg N/plant were obtained for cv. Giza 5, respectively. It is, therefore, suggested that in addition to rhizobial inoculation, the treatment with cyanobacterial filtrates as a source of phytohormones led to significant enhancement of both growth and nodulation of peanut.

Key words: *biofertilization, cyanobacteria, legumes, peanut, rhizobia.*

1. INTRODUCTION

Peanut (*Arachis hypogaea* L.) is considered one of the most important oil seed leguminous crops and cultivated regularly in sandy soils in Egypt.

Several reports described the potential uses of plant associated bacteria as agents stimulating plant growth and managing soil and plant health (Hallman *et al.*, 1997 and Sturz *et al.*, 2000). Plant growth promoting rhizobacteria "PGPR" (Bashan and Holguin, 1998) are associated with many, if not all, plant species and are commonly present in many environments. Consequently, intimate associations between bacteria and a host plant can be formed without harming the plant (Klopper and Beauchamp, 1992, and Lodewycky *et al.*, 2002).

Co-inoculation of legumes with PGPR and *Rhizobium* has recently received increasing attention and can produce plant growth regulators. Dual inoculation with both symbiotic and asymbiotic bacteria may increase nodulation through a variety of mechanisms. For example, PGPR were reported to colonize plant root surfaces, produce phytoalexin, antibiotics and siderophores (Parmer and Dadarwal, 1999 and Postma *et al.*, 2003). Additionally, dual inoculation with *Rhizobium* and PGPR can

stimulate or inhibit nodule formation and growth in a given symbiotic relationship, depending upon the nature and concentration of secondary metabolites released by the non-rhizobial PGPR.

On the other hand, cyanobacteria were reported to produce several bioactive substances that affect plant growth, such substances may include gibberellic acid (GA), indole acetic acid (IAA) and abscisic acid, ABA (Zaccaro de Mule *et al.*, 1991 and Yavorska and Dragovoz, 2001).

This study was conducted to point out the influence of single and dual rhizobial inoculation of peanut (*Arachis hypogaea* L.), cvs. Georgia and Giza 5, with KH 8 and KH 11 strains. It has been done in presence of cyanobacteria. In this respect, plant parameters and nodulation were evaluated at the end of the experiments.

2. MATERIALS AND METHODS

2.1. Host plants

Seeds of peanut (*Arachis hypogaea*) cvs. Giza 5 and Georgia were kindly provided by Legume Crops Department ARC., Giza. Each cultivar is recommended for cultivation in its representative region. Seeds were selected to be similar in size and weight to the nearest 10 mg.

2.2. Rhizobial strains

Twelve isolates of *Rhizobium* sp. specific to peanut were obtained from root nodules of peanut cultivated at the ARC Research Station in Noubaria and Badr city. Rhizobial isolates were maintained on yeast extract mannitol (YEM) agar medium (Somosegaran and Hoben, 1994), and were tested for their effectiveness applying plant infection technique (Vincent, 1970).

2.3. Cyanobacterial strains

Three filamentous heterocystous cyanobacterial strains belonging to the genera *Anabaena*, *Nostoc* and *Calothrix* were kindly obtained from the Department of Agricultural Microbiology, Fac. Agric., Cairo University (Higazy *et al.*, 2004) and were applied in some treatments. All cultures were maintained on Allen and Arnon medium (Allen and Arnon, 1955) under phototrophic growth conditions. Stock cultures were grown under continuous illumination, with Philips florescent white lamps at a relatively low light intensity (400-500 lux) and incubated at 30°C. GA₃, IAA and ABA were determined in culture filtrates of these strains by Gas Liquid Chromatography (Bye Unicam pro-GLC) according to the method described by Vogel (1975) in the Central Laboratory, Faculty of Agriculture, Cairo University. Data were expressed as quantity of phytohormones as g 100 ml⁻¹ culture filtrate⁻¹.

2.4 Preparation of cyanobacterial filtrate

A filtrate mixture of 1:1:1 was prepared from a 30-day old cultures of either *Anabaena*, *Nostoc* or *Calothrix*. Fifty ml of this mixture were added on soil surface before and after 15 days of seed germination.

2.5. Soil analysis

The soil used in pot experiment was collected from a new reclaimed region at Ismailia Governorate. The soil properties were determined according to Piper (1950) and Jackson (1973) and are presented in Table (1). Soil samples were taken from the top 0-30 cm, air dried and distributed in plastic pots (φ 30 cm) at the rate of 10 kg pot⁻¹.

2. 6. Preparation of rhizobial inocula

Two hundred ml of YEM liquid medium (Vincent, 1970), distributed in 500 ml conical flasks, were inoculated with *Rhizobium* sp. strains and incubated on a rotary shaker for 5-7 days at 28°C. In the greenhouse experiment, each seed was inoculated with 1 ml (ca. 10⁹ cells) from the rhizobial culture.

2.7. Experimentation

2.7.1 Plant infection experiment

Two plant infection technique experiments were made using Linard jars system (Vincent, 1970) to select the potent strains that interacted with peanut cvs. Georgia and Giza 5. At the end of the experiment, fresh and dry weights of both shoots and roots as well as nodulation parameters were determined.

2.7.2 Pot experiment

Pots of 30 cm diameter were filled with 10 kg soil portions. In all treatments, soil was amended with ammonium sulphate (NH₄)₂ SO₄ (20.5% N) at the rate of 25 kg fed⁻¹ as a starter dose before sowing. Phosphate fertilizer was incorporated into soil at the rate of 200 kg fed⁻¹ as super phosphate (15.5% P₂O₅). Seeds of peanut were sown at the rate of 5 seeds pot⁻¹. Three replicates were used for each treatment. After 45 days of planting, plants were uprooted and determined for shoot and root dry weights (mg plant⁻¹), after drying at 70°C, shoot N-content as well as the number and biomass (mg plant⁻¹) of nodules were also determined.

Nine treatments were applied as follows:

- 1- Control 1 (without N fertilization or inoculation),
- 2- Control 2 (recommended dose of N without inoculation),
- 3- Rhizo inoculation with strain Kh 8.
- 4- Rhizo inoculation with strain Kh 8 + cyano,
- 5- Rhizo inoculation with strain Kh 11.
- 6- Rhizo inoculation with strain Kh 11 + Cyano.
- 7- Rhizo inoculation with strain kh 8 + K 11.
- 8- Rhizo inoculation with strain kh8 + k 11 + Cyano.
- 9- Cyano treatment only.

Table (1): Mechanical and physicochemical properties of Ismailia soil used in the greenhouse experiments.

Properties	Values
Coars sand (%)	51.14
Fine sand (%)	45.14
Silt (%)	1.30
Clay (%)	2.20
Textural class	Sandy
Organic-carbon (%)	0.11
Organic matter (%)	0.19
Water holding capacity	16.11
pH	7.91
E.C. (d sm ⁻¹)	0.30
Soluble cations (meq/l):	
Ca ⁺⁺	0.72
Mg ⁺⁺	0.50
Na ⁺⁺	1.60
K ⁺⁺	0.14
Soluble anions (meq/l):	
HCO ₃ ⁻	1.77
CL ⁻	0.60
SO ₄ ⁼	0.59
CO ₃ ⁼	0.00

3. RESULTS AND DISCUSSION

3.1 Screening of rhizobia isolates for inoculation experiments

Twelve strains of *Rhizobium* sp. were screened for their effectiveness with two peanut cultivars. The data in Table (2) indicated that the highest nodule numbers of 228 and 241 plant⁻¹, nodule dry weights of 0.44 and 0.51 mg plant⁻¹ were obtained for peanut cv. Georgia when being inoculated with Kh8 and Kh 11, strains respectively. Table (3) presents the highest nodule numbers of 252 and 266 plant⁻¹, nodule dry weights of 0.51 and 1.1 mg plant⁻¹ were obtained for peanut cv. Giza 5 after being inoculated with Kh 8 and Kh 11, strains in that order. Therefore, the strains Kh8 and Kh11 proved to be the most efficient and subsequently were selected for the pot experiment.

3.2 Growth and nodulation of peanut cv. Georgia due to rhizobial inoculation and cyanobacteria application.

Data in Table (4) indicate that significant increases, in response to the various treatments, were observed. For example, the highest increases in shoots nitrogen content, roots dry weight and nodules dry weight were 64.2, 666 and 920% over their corresponding controls, respectively in treatments 9, 8 and 8, in that order. Also, it is an interesting observation that when cyanobacterial filtrate was introduced with either Kh 8 or Kh 11, individually or in mixture, peanut plants exhibited higher values in their growth and nodulation parameters. Their shoot nitrogen content, root dry weight and nodule dry weight reached 99 mg plant⁻¹, 4.6 mg plant⁻¹ and 0.51 mg nodule⁻¹, respectively.

3.3 Influence of rhizobial inoculation and cyanobacterial application on the growth and nodulation parameters of peanut cv. Giza 5.

Similar results were found with peanut cv. Giza 5 and demonstrated in Table (5). It could be concluded that the response of peanut plants to rhizobial inoculation was much better when cyanobacterial filtrates were introduced. For instance, the highest records of shoot nitrogen content, root dry weight and nodule dry weight were 148.15 mg plant⁻¹, 1.79 mg plant⁻¹ and 0.49 mg nodule⁻¹, respectively, were obtained in treatments 9,3 and 8, in that order.

3.4. Cyanobacteria growth promoting regulators

The ability to produce some growth promoting substances, *i.e.*, phytohormones was examined for all the tested cyanobacterial strains. Table (6) shows that *Nostoc* sp produced 0.3012 g indole acetic acid (IAA) 100 ml⁻¹ culture filtrate⁻¹. While, the same strain was able to excrete 0.8005g abscisic acid (ABA) 100 ml⁻¹ culture filtrate⁻¹. Also, *Anabaena* sp did produce 3.010 g gibberellic acid (GA₃) 100 ml⁻¹ culture filtrate⁻¹.

The previous observations may indicate that both rhizobial inoculation and cyanobacteria application increased the root, shoot and nodule dry weights. In this respect, Dey *et al.* (2004) reported that both growth and yield of peanut (*Arachis hypogaea* L.) were enhanced by the application of plant growth-promoting rhizobacteria.

Nasef *et al.* (2006) studied the effect of foliar spray with boron and *Rhizobium* inoculation on peanut plants grown in sandy soil. They found that boron foliar spray at 220 ppm in combination with seed inoculation with *Rhizobium* enhanced plant growth and improved peanut yield and its components. Cyanobacteria were reported to have many useful applications such as improving the growth, development and biofertilization of many plants (Malliga and Subramaniam, 2002), production of plant growth promoting substances like IAA and ABA (Yavorska and Dragovoz, 2001). Therefore, it is not surprising that the application of *Nostoc*, *Anabaena* and *Calothrix* as a source of phytohormones in this study, led to significant increases in peanut growth and nodulation parameters.

Regarding the plant growth promoting rhizobacteria (PGPR) and stimulation of legume – rhizobia symbioses, it is evident that the most commonly implicated mode of action is the stimulation of root growth (Molla *et al.*, 2001 and Vessey and Buss, 2002), which may provide more sites for infection and nodulation. Some PGPR may increase the availability of nutrients for the plant in the rhizosphere (Rodriguez and Frage, 1999) and increase root surface area (Antoun *et al.*, 1998).

Table (2): Influence of inoculation with several rhizobial isolates on the growth and nodulation of 45-day old plants of peanut cv. Georgia.

Treatments	Shoots		Roots		Nodules		
	FW*	DW	FW*	DW	No.	FW	DW
	(mg plant ⁻¹)		(mg plant ⁻¹)		(mg nodules ⁻¹)		
Control	20.13	4.73	10.50	1.30	88	0.10	0.20
Kh ₁	26.53	6.43	15.96	1.93	146	1.43	0.29
Kh ₂	18.63	4.60	13.33	1.26	98	0.73	0.18
Kh ₃	22.83	4.03	14.43	1.43	181	0.57	0.37
Kh ₄	23.16	5.60	16.60	1.60	144	1.20	0.31
Kh ₆	20.16	4.73	12.40	1.31	153	0.50	0.28
Kh ₇	23.33	5.27	14.10	1.33	228	1.23	0.42
Kh ₈	23.56	5.66	15.40	1.42	228	2.36	0.44
Kh ₉	26.37	6.20	14.63	1.60	165	1.63	0.36
Kh ₁₀	17.86	4.06	19.66	0.86	163	1.26	0.27
Kh ₁₁	16.14	5.53	16.10	1.65	241	2.13	0.51
Kh ₁₂	25.23	5.93	14.20	1.61	197	1.50	0.40
Kh ₁₄	21.33	4.83	13.07	1.33	209	2.03	0.44
L.S.D.	2.91	0.69	1.80	0.23	37.4	0.34	0.06

Fw, fresh weight; Dw, dry weight; No., numbers.

Table (3): Growth and nodulation parameters of 45-day old plants of peanut cv. Giza 5 after inoculation with several strains of rhizobia.

Treatments	Shoots		Roots		Nodules		
	FW*	DW	FW*	DW	No.	FW	DW
	(mg plant ⁻¹)		(mg plant ⁻¹)		(mg nodules ⁻¹)		
Control	15.3	4.3	10.1	1.6	11	0.10	0.01
Kh ₁	27.8	6.8	16.3	1.6	176	1.50	0.29
Kh ₂	26.0	6.5	16.9	1.4	209	1.40	0.36
Kh ₃	21.0	4.7	10.4	0.9	142	1.40	0.32
Kh ₄	23.2	5.4	19.3	2.1	81	0.50	0.20
Kh ₆	18.1	4.3	12.1	1.1	169	0.70	0.31
Kh ₇	23.7	5.5	13.4	1.2	165	1.70	0.46
Kh ₈	20.9	4.9	19.2	1.3	252	2.25	0.51
Kh ₉	22.2	5.3	16.3	1.21	108	1.30	0.22
Kh ₁₀	19.4	4.3	9.1	0.7	65	0.70	0.13
Kh ₁₁	22.2	5.1	18.7	1.9	266	2.55	1.10
Kh ₁₂	19.4	4.5	14.4	1.7	198	1.60	0.38
Kh ₁₄	18.6	4.2	9.9	0.9	196	2.10	0.40
L.S.D.	2.52	0.71	1.80	0.33	31.9	0.43	0.053

Fw, fresh weight; Dw, dry weight; No., numbers

Table (4): Effect of rhizobial biofertilization and cyanobacterial application on growth and nodulation of 45-day old plants of peanut of cv. Georgea

Treatments	Shoots			Roots		Nodules		
	FW*	DW	N	FW	DW	No.	FW	DW
	mg/plant			mg/plant		mg/nodules		
1. Control (1)	32.30	8.6	62.20	1.50	0.60	11	0.10	0.05
2. Control (2)	19.93	5.0	54.03	1.16	0.93	4	0.10	0.04
3. Kh8	27.60	7.4	53.90	13.80	1.03	110	0.87	0.20
4. Kh8+Cyano	27.70	7.4	69.70	11.90	2.60	127	1.66	0.29
5. Kh11	25.80	6.1	67.63	15.20	1.30	147	1.18	0.20
6. Kh11+ Cyano	24.13	7.1	85.16	17.90	1.70	166	1.38	0.25
7. Kh8+Kh11	26.70	6.8	84.00	14.50	1.80	174	1.17	0.23
8. Kh8+Kh11+ Cyano	36.13	8.7	99.00	21.90	4.60	181	1.66	0.51
9. Cyano only	41.90	10.7	102.10	21.70	1.90	117	1.25	0.45
L.S.D. 0.05%	4.47	1.10	9.72	1.84	0.78	17.49	0.145	0.052

FW fresh weight; DW, dry weight; No., numbers, N, nitrogen content

Table (5): Growth and nodulation parameters of 45-day old plants of peanut cv. Giza 5 after biofertilization with different rhizobial strains and cyanobacteria application.

Treatments	Shoots			Roots		Nodules		DW
	FW	DW	N	FW	DW	N o.	FW	
	mg/plant			mg/plant		mg/nodules		
1. Control (1)	22.33	6.2	67.5	10.5	0.97	23	0.20	0.02
2. Control (2)	17.50	5.1	52.6	9.1	0.83	6	0.10	0.07
3. Kh8	22.30	6.2	67.5	10.5	1.97	33	1.20	0.09
4. Kh8+Cyano	23.70	5.4	130.9	12.7	1.50	150	1.40	0.31
5. Kh11	26.50	4.9	107.7	10.4	1.13	118	1.27	0.31
6. Kh11+ Cyano	26.63	7.0	99.2	12.4	1.40	147	1.50	0.41
7. Kh8+Kh11	19.96	4.3	12.6	9.8	1.13	191	1.63	0.38
8. Kh8+Kh11+ Cyano	20.10	4.4	146.1	16.5	1.10	219	1.33	0.49
9. Cyano only	25.20	5.4	148.2	11.9	1.10	155	1.34	0.45
L.S.D. 0.05%	3.21	0.64	16.7	1.70	1.22	11.88	0.26	0.047

* Fw, fresh weight; DW, dry weight; No., numbers, N, nitrogen content.

Table (6): Phytohormones composition in cyanobacterial cultures filtrates.

Cultures	IAA*	ABA	GA ₃
	(g/100ml ⁻¹ filtrate ⁻¹)		
Anabaena sp.	0.2510	0.1862	3.010
Nostoc Sp.	0.3012	0.8005	2.415
Calothrix sp.	0.1688	0.1005	1.825

* IAA, indole acetic acid; ABA, abscisic acid; GA₃, gibberallic acid.

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تحسين نمو وتعقيد الفول السوداني بالتلقيح بالريزوبيا والمعاملة بالسيانوبكتريا

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ملخص

تناولت الدراسة تقييم استجابة الصنفين من الفول السوداني جورجيا و جيزة 5 فى تجربة أصص للتلقيح بالريزوبيا مع المعاملة براشح السيانوبكتريا كمصدر لبعض الهرمونات المنظمة للنمو. أظهرت النتائج ان الوزن الجاف لكل من جذور و سيقان نباتات الفول السوداني قد زاد معنوياً نتيجة التلقيح بسلالتي الريزوبيا Kh 8 , Kh 11 . كذلك أوضحت النتائج أن المعاملة براشح بعض سلالات السيانوبكتريا قد أدى الى تشجيع نمو و تعقيد نباتات الفول السوداني حيث أظهرت النباتات أعلى القيم فى الوزن الجاف للجذور (1.93 مجم/ نبات) والسيقان (6.43 ملجم / نبات) وكونت أكبر عدد من العقد الجذرية (240 عقدة / نبات).

من جهة أخرى، أثبتت النتائج أن المحتوى النتروجينى لنباتات الفول السوداني للصنفين المختبرين زاد نتيجة التلقيح بالسلالتين Kh 8 , Kh 11 والمعاملة براشح السيانوبكتريا حيث وصلت هذه القيم الى 99 ، 146مجم / نبات فى صنفى جورجيا و جيزة 5 على الترتيب. وقد انتهت الدراسة الى أن التلقيح بالريزوبيا بجانب المعاملة بالسيانوبكتريا يمكن ان يؤدي الى تحسين كل من نمو و تعقيد نباتات الفول السوداني.

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