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Effect of N-Rate and P Sources on BNF in Soy bean as Affected by Rhizobium and VAM Fungi Inoculants

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خلاصة

أجري هذا البحث في الصويه الزراعية لدراسة تأثير مصادر الفوسفات على تثبيت الآزوت في فول الصويا، وقد استخدمت طريقة التخفيف النظائري للنظير الثابت (ن - 15) لتقدير كمية النيتروجين المثلث. ثم معاملة بذور صنفين من فول الصويا (الذي يكون عقد جذرية والذي لا يكون عقد جذرية) الـ Rhizobium Japonicum وقسمت الأصص إلى قسمين عومل الأول بفطريات الميكوريزا وترك الآخر دون معاملة تمت كل هذه المعاملات تحت إضافة كل من مصدري الفوسفات (السوبر فوسفات - صخر الفوسفات) هذا وقد أضيف السماد النيتروجيني المرقم (ن - 15) في صورة كبريتات الأمونيوم بمعدلين 25 كجم/ ايكر (5% N-15 a.e). أوضحت النتائج أنه في مرحلة التزهير كانت كمية النيتروجين المأخوذ من الهواء الجوي 390.1 و290.23 مجم/ إصيص والتي تعادل 40.94٪ و34.814٪ من النيتروجين الكلي في النباتات المعاملة بالريزوبيوم والمسمدة بالسوبر أو الصخر الفوسفاتي على التوالي بينما كانت كمية النيتروجين المأخوذ من الارض لنفس المعاملة السابقة كانت 10.85٪، 15.39٪ . كما أظهرت النتائج أن إستخدام الميكوريزا يؤدي إلى زيادة تثبيت الأزوت الجوي في وجود الريزوبيوم ويبدو من النتائج أن استخدام التلقيح المزدوج بكل من الريزوبيوم والميكوريزا ذو أثر فعال في تثبيت الأزوت الجوي خاصة في الأراضي الرملية.

Abstract

Greenhouse experiment was made to investigate the influence of phosphate fertilizers on nitrogen fixation in soybean. The N-15 isotope dilution method was used to quantify N_2-fixed. In this concern, seeds of nodulated and non-nodulated soybean plant bacterized with Bradyrhizobium Japonicum and inoculated without or with mycorrhizae in the

presence of super or rock phosphate. Ammonium sulphate labelled fertilizer (5 and 3% N-15 a.e) was applied to 15 kg Egyptian sandy soil at the rate of 20 and 100 kg N/acre.

At pre-flowering stage, the highest amount of N derived from air (Ndfa) was 566.3 and 470.2 (mg/pot) equivalent to 47.6 and 47.1 of total N assimilated for inoculated soybean with Rhizobium and fertilized with super or rock phosphate, respectively. While the contributions from ^{15}N labelled fertilizer (Ndff) accounted for 11 and 10.8, respectively. Use of mycorrhizae could increase the amount of N2-fixed in the presence of rhizobia. There appears to be a strong case for improving N2-fixation in the presence of mycorrhizae especially in sandy soil .

Introduction:

Legumes have the capacity of derive a considerable proportion of their N requirements from the atmosphere through symbiosis with Rhizobium. The propotion derived is dependent upon the host species, strains of Rhizobium, and environment interactions. Under favourable conditions, soybean can derive as much as 40-60% of their N requirements through N2-fixation [17]. Hence, it may be necessary to apply supplementry N especially to legumes such as soybean to realize maximum yield [2]. In Egypt, the amounts of atmospheric nitrogen fixed by soybeans plants,which are newly introduced, have been found to be low.

Phosphorus plays a key role in biological nitrogen fixation (BNF). For the symbiotic nitrogen fixation, the roots have to interact with compatible rhizobia in the soil, and factors that affect root growth or the activity of the host plant would affect nodulation [14]. Nodule formation, bacterial growth and the BNF activity itself are processes that are dependent on the energy supplied from the sugars that need to be translocated downward from the plant shoots [15]. Phosphorus is the basis for formation of useful energy which is essential for sugar formation and translocation.

Soybean enters into one type of symbiotic association with the bacteria of the genus Bradyrhizobium and into another with vesicular arbuscular mycorrhizal (VAM) fungi that improve nutrition of plants [8]. It has been shown that a dual inoculation of soybean by VAM fungi and Rhizobium bacteria under various conditions improves the growth and yield of plants more than the inoculation with one of them only. The contribution of VAM fungi to dual symbiosis is related to high P requirement for nodulation and N2-fixation [6].

The main goal of this work is to:

I) determine the beneficial of VAM in the presence of P sources and N levels on growth, nodulation, spore No., root infection, N and P uptake by inoculated soybean with Rhizobium.

II) to specify the impact of Rhizobium and VAM fungus on N derived from fertilizer (Ndff) and air (Ndfa) as well as N2-fixation using N-15 technique.

2. Material and methods

This experiment was carried out at the greenhouse of Soils & Water Dept., Nuclear Res. Center, Atomic Energy Auth. Cairo, Egypt. Sandy soil in texture was collected from the area of the research farm of AEA Inshas. The preliminary analysis of the soil used showed 0.1% organic matter, 0.0004% total N, 3 ppm available P and pH 7.9. Nodulating soy bean seeds (Glycine max (L.) Merr. CV Clark) and two levels of 5N labelled ammonium sulphate fertilizer, 20 mg N.Kg soil of 5% N atom exess and 100 mg N.Kg soil of 3% N at.ex. were used in the presence of 25 mg P Kg/l soil as super or rock-phosphate. An effective strain of Bradyrhizobium Japonicum (Rh) strain 110 was obtained from Agric. Microbiol. Sec., Soil & Water Inst., ARC, Giza, Egypt. A standard inoculum was prepared by adding manitol broth, and cell suspension are calibrated to give final concentration of 10° cell ml^{-1}. Seeds were soaked in B. Japonicum for 12 hr before planting. A none-nodulating soybean isoline (0108) was used as reference crop for determining N2-fixation. Two hundred grams of mycorrhizal (VAM) inoculum produced in soy bean pot culture and consisting of a root soil fungus mixture containing 5000-6000 spores,blended into the central third of the sand soil.

Pots (35 cm x40 cm) were filled with 15 kg of soil. Six seeds were planted per pot, and after germination, they were thinned out to four per pot. Super or rock phosphate was applied before planting. Labelled fertilizer was applied in solution. Experiment was factorial and arranged in a randomized complete block design with three replicates. During the experimental period, pots were kept weed free and watering was done when necessary.

After 70 days, plants were harvested, Roots were removed to determine nodule number and dry weight. The colonization of roots by VAM fungi was measured and the spore number in each treatment was detected. Shoots dry weight were recorded after drying at 65°C. Total N and P analysis in ground samples were determined by standard analytical method and $^{15}N/^{14}N$ ratio measurements using an emission spectrometer. Proportions and amounts of N derived from atmosphere were calculated by 15N isotope dilution technique according to [16].

Results and Discussion

Dry weight of shoots and nodules

Data presented in Table (1) show the effect of inoculation, N-rates and P sources on shoot growth and nodules dry weight of soybean plant at pre-flowering (70 days). Shoots dry weight of nodulated and non-nodulated isoline soybean significantly increased with increasing N levels from 20 to 100 mg N/kg soil in the presence of either super or rock phosphate. Weber [26] reported that yield of nodulating and non nodulating varieties was practically the same when mineral-N was supplied, at increasing levels of N.

Concerning, nodules dry weight, results in the same table showed that significantly increasing N levels, reduced nodules dry weight of soybean plant. These results are in agreement with those obtained by [1]. They found that application of small quantities of N-fertilizers favoured nodules formation, while, large quantities of N adversely affected nodulation. Brockwel et al. [9] added that with high levels of mineral N,the colonization of seedling rhizospheres by rhizobia and plant nodulation were diminished.

The data also show that dry weight of both shoots and nodules were significantly affected by inoculation with Rhizobium (Rh) and/or mycorrhizae (VAM) in the presence of either super or rock-P. In this respect, soy bean infected with Rhizobium and fertilized with super or rock-P significantly increased shoot growth by about 26 and 33% over uninoculated control plants, respectively. Also, data showed that plants inoculated with VAM and Rhizobium were 12 and 13% greater in dry weight than plants inoculated with Rhizobium only and when plants supplemented with super or rock-P, respectively. This increase was due to a positive VAM X Rhizobium interaction. The better response of soybean to Rhizobium inoculation could be attributed to the complete absence of soybean specific native rhizobia in the sandy soil used. Ross and Harper [24] recorded 29% increase in yield of soybean grown in fumigated field plots, due to double inoculation with endomycorrhizae plus Rhizobiumover single inoculation with only Rhizobium Also, Kawai and Yamomoto [20], reported that the increase in dry matter content of legume plants is a result of dual inoculation with Rhizobium and mycorrhizal due to stimulative effect on nodulation and N2-fixation.

The present data showed that VAM fungal colonization alone had no significant effect on both shoots and nodules dry weight.These results are in accordance with that obtained by [19], who found that there was no effect of mycorrhizal inoculation on soybean yields under natural field conditions.

Moreover the present, data showed that higher rates of increase in dry matter occurred with rock-phosphate supplement rather than with super phosphate application. This case might indicate an apparent favour for rock-P upon super-P, which is completely controversial to the absolute values of dry matter increments, as a function of inoculants whether in the presence of super or rock-P.

With regard to nodules dry weight, results in Table (1) indicate that the application of both super or rock-P significantly improved the weight of root nodules of soy bean.

The maximum nodulation was recorded when plants were inoculated with Rhizobium plus VAM followed by Rhizobium alone under different P sources. However, the superiority was more pronounced for rock-P than super-P. Pacovsky and Fuller [23] reported that Nodulation and N2-fixation are highly dependent on P uptake, and the presence of VAM fungus increased P acquisition. They added that the effectiveness of VAM fungi is greatest in soils amended with sparingly soluble P, and concluded that rock-P addition greatly enhanced nodulation and nitrogen fixation of the mycorrhizal plants which were inoculated with the appropriate Rhizobium strain. Regarding the interaction between N.x.P.x inoculants, the results showed that increasing N rates had a remarkable increase on dry matter content. On the other hand, highest dry matter contents were obtained when plants were inoculated with Rhizobium alone or combined with mycorrhizae at 20 mg N/Kg^{-1} soil in the presence of super or rock phosphate. In this respect, the net increments in dry matter content were 33 and 48% of uninoculated control treatments when plants inoculated with Rhizobium alone and dual inoculation at 20 mg N/Kg^{-1} in the presence of super-P, respectively. While, with rock-P application, it amounted to 41 and 67% over control for respective inoculants.Data presented in Table (1), show that dual inoculation was more effective for increasing nodules dry weight at 20 mg N/Kg soil in the presence of super or rock-P. In this case, it recorded 689.2 and 722.4 mg/pot for respective treatments. While, at 100 mg N/Kg^{-1} soil, it reached to 372.2 and 391.2 mg/pot for the previous treatments, respectively. Mosse [22] explained this synergistic effect between VA mycorrhizal fungi and rhizobia. She suggested that while the principal effect of mycorrhizae on nodulation is undoubtedly phosphate mediated, mycorrhizae may have other secondary effects, possibly of hormonal nature.

Spore number and root infection

Regarding, spore number and root infection, data presented in Table (2) showed that sporulation was found to be affected favourably with rhizobial inoculation either in the presence of super or rock-P, VAM infection percent was almost not responded possibly due to the relatively low viable efficiency of such existing spores. The increase in sporulation could be due to an enhancement of root exudates which may affect the growth of soil flora, particularly when rhizobia are introduced to the plant. Similar results were observed by [18].

The present, data indicated that infection with VA mycorrhizae alone or in combination with Rhizobium had a great effect on both spore density and VAM infection percent under fertilization with super or rock-P. This goes along with the results obtained by [5]. They suggested that rhizobia enhance the mycorrhizal establishment by producing polysacchardes leading to increase in synthesis of poly galcturonase enzyme at the infection site. This enzyme being able to facilitate the permeability of root cells to fungus.

Inoculation with Rhizobium accompanied with VAM appeared to be more effective at 20 ppm N level regarding sporulation and VAM infection percentage in the presence of either super or rock-P (Table 2). Gewaily and Kheder, (1985) [14] observed that the influence of mineral N fertilization on VAM is dependent on soil type with relatively high N-level (200 kg ha^{-1}) being depressant in sandy loam soil for VAM infection but hardly influencing in silty loam one. Louis and Lim [22] added that combination between rhizobia and mycorrhizae was a promotive factor on mycorrhizal development and spore population in soil even after addition of a moderate dose of P to soil.

N and P uptake

The N and P content of the shoot at two N levels are presented in Table 3. Results showed that nitrogen and phosphorus content of the plants inoculated with Rhizobium + VAM were significantly higher as compared with uninoculated plants and plants which inoculated only Rhizobium or VAM in the presence of super or rock-P. In this respect, the percentage rate of increases were 57 and 69% over control for N; uptake 60 and 77% for p uptake upon fertilization with super or rock-P after plant inoculation with VAM + Rhizobium. For, inoculation with Rhizobium or VAM alone, the rates of increase in N uptake in the presence of super-P were 37 and 23%

control; 33 and 77% upon fertilization with rock-P for the previous treatments, respectively. These results are in agreement with these obtained by [24]. They reported that increased shoot N and P in the tripartite association can lead to increased levels of photosynthesis that could be capable of supplying the increased C requirements of the microsymbionts.

The present, data showed that the VA mycorrhizal inoculation was less favourable for N content as compared with effects obtained with rhizobia. Effect of mycorrhiza on plant N was generaly clear when mycorrhizal is associated with nitrogen- fixing plants. This is due to the favourable effect of VAM on nodulation and nitrogenase activity in nodules [17].

Different trend was encountered with phosphorus whose concentration in infected plants significantly increases as compared with nonmycorrhizal plants. Such responses were explained by [11] on the basis of relatively higher activity of VA mycorrhizal hyphae to absorb soil P and its translocation to the host roots through a specific efficient active mechanism.

Under conditions of nitrogen fertilization, the association between rhizobia and mycorrhizae appeared to be significantly favourable for development on N and P uptake by shoots of soybean plants.

Proportion of plant nitrogen

Nitrogen derived from fertilizer (Ndff).

It is clear from the results obtained in Table 4 that at 20 mg M.Kg soil, seed inoculation with Rhizobium and/or mycorrhizae resulted

in a remarkable decrease in the proportion of its N derived from fertilizer as compared with the uninoculated or non nodulated plants. Plants given 100 mg M. Kg/1 soil showed a remarkable higher proportion of fertilizer N derived than those given only 20 mg N.Kg soil.

In this regard, shoots of soybean plants accumulated about 10 to 18 and 42 to 56% of fertilizer N at 20 and 100 mq N.Kg soil respectively under various inoculants in the presence of super or rock-P. These results are in good agrement with that obtained by [15].

The present data showed as well, that there was no difference in effect between the two difference P sources on Ndff%.

Percent N derived from air (% Ndfa)

Data presented in Table (4) showed that about 48% of the N in the nodulated soybean was derived from atmosphere (%Ndfa), when plants were inoculated with Rhizobium only and fertilized with 20 mg N.Kg soil as ammonium sulphate in the presence of super or rock-P. Application of 100 mg N.Kg soil reduced nitrogen fixation to about 18% Ndfa. Thus, the higher levels of N2-fixed were obtained when the %Ndff was low which suggests that the fertilizer reduced the symbiotically fixed N through. Results showed that the amounts of N2-fixed by soybean inoculated with Rhizobium alone gave about 566.3 and 470.2 mg N Kg^{-1} soil at 20 mg N Kg soil in the presence of either super or rock-P,respectively; While, it was reduced to 189 and 170 mg N Kg^{-1} soil at 100 ppm N added to soil for the previous treatments. Data showed that dual inoculation (Rhizobium + mycorrhizae) gave the highest amount of N_2 at 20 mg N Kg^{-1} soil (689.1 mg/pot) when supplemented with super and 643 mg/pot in the presence of rock-P. Increased N fertilization up to 100 mg N Kg^{-1} soil resulted in a remarkable decrease in the amount of N_2 fixation. This was reduced to 311 and 296 mg N Kg upon fertilization with super or rock-P, respectively. The same trend was also obtained by [10].

The present results seem to provide experimental support for the common field practice of supplying a small quantity of N fertilizer as a starter for the crop to support intial growth before N_2-fixation can become significant. Alaides et al.[3] added that small amounts of combined-N seem to have a valuable synerigistic effect in relation to biological N_2-fixation. Giving too much nitrogenous fertilizer is however self-defeating as it did not increase the total level of nitrogen in the system, but merely led to reduced N_2-fixation.

It is also interesting to note that more Ndfa was obtained when soybean plants were colonized with both VAM fungus and Rhizobium (Table 4). In this concern, %Ndfa was markedly increased by about 8 and 12% when plants were colonized with dual inoculation compared with Rhizobium alone upon fertilizing with super and rock-P at 20 mg $N.Kg^{-1}$ soil, respectively. These finding imply additive or synergistic relationship between microorganisms [7]. Results are in accordance with that obtained by [6]. They reported that the contribution of VAM fungi to dual symbiosis is related to a high phosphorus requirement for nodulation and N_2-fixation. The present, data showed that, at 100 mg $N.Kg^{-1}$ soil, %Ndfa was reduced to 25 under both super-or rock-P. The lesser amount being associated with higher proportions of soil and fertilizer N levels.

Amounts of N_2-fixed

Amount of nitrogen fixed was markedly affected by N rates, P sources and inoculants (Table 4). VA mycorrhizal fungus could contribute to the efficiency of such a system, especially in phosphorus-deficient soils, even though native endophytes may be present. Generally, it could be noticed that soybean inoculation with Rhizobium alone or in mixture with mycorrhizae and combined with an activation dose of nitrogen (20 Kg N fed^{-1}) in the presence of super or rock-P, always support greater activity as compared with uninoculated control or application of the higher nitrogen dose plus inoculants. In this respect, the amounts of N_2-fixed were significantly increased from 566.3 to 689.1 mg Pot^{-1} in the presence of super P and fertilization with 20 Kg N fed^{-1} when plants, were inoculated with Rhizobium alone or combined with mycorrhizae. In the presence of rock-P, it ranged from 470.2 to 642.7 mg N Pot^{-1} for respective treatments. Moreover, data showed that inoculation along with application of high rate of nitrogen fertilizer markedly decreased the amounts of N_2-fixed. It is clear that low rate of combined nitrogen may result in increasing plant growth and symbiotic N_2-fixation presumably by giving the plant an early booster of nitrogen, which makes it healthier to support the N_2-fixation symbiosis [12].

The results may show that rhizobia alone or combined with VA mycorrhiza and conjugated with the proper amount of mineral nitrogen is the practice to be adopted in Egyptian agriculture. The cheap rice of Rhizobium inoculates may represent another reason to support the concept of inoculation as a common practice especially in new reclaimed sandy soil.

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TABLE 1: EFFECT OF N RATE APPLICATION ON DRY MATTER, NODULES DRY WEIGHT OF SOY BEAN PLANTS AS AFFECTED BY P-SOURCES AND RHIZOBIUM AND/OR MYCORRHIZAE

Treatments	dry weight (g/pot)			Nodules weight (mg/pot)		
	20	100	Mean	20	100	Mean
	mg N kg^{-1} soil			mg N kg^{-1} soil		
Super -P						
Uninoculated	23.73	28.23	25.98	0.76	0.32	0.54
(Rh)	31.52	33.98	32.75	531.62	311.13	421.38
(VAM)	26.91	29.33	28.12	1.14	0.34	0.74
Rh + VAM	35.06	38.21	36.64	689.24	372.24	530.74
Mean	29.31	32.44		305.69	171.0	–
None nod.	24.26	29.22	26.74	–	–	–
Rock-P						
Uninoculated	19.18	26.11	22.65	0.61	0.11	0.36
Rh	27.05	33.22	30.14	616.53	351.42	483.98
VAM	23.11	27.13	25.12	1.21	0.20	0.71
Rh + VAM	32.09	36.17	34.13	722.35	391.41	556.88
Mean	25.36	30.65		–	–	–
Mone nod.	21.94	27.61		335.18	185.79	–

	P Sources	N rate	I	PxI	NxI	PxNxI
Drymatter	4.23	2.52	2.43	1.09	1.36	N.S
Nodules weight	110.26	110.26	55.44	49.11	51.45	22.36

TABLE 2: EFFECT OF N RATE APPLICATION ON SPORE AND ROOT INFECTION OF SOY BEAN PLANTS AS AFFECTED BY P SOURCES AND RHIZOBIUM AND/OR MYCORRHIZAE.

Treatments	Spore No. (No/pot) x10-3			R.I %		
	20	100	Mean	20	100	Mean
	(mg N kg^{-1} soil)			(mg N kg^{-1} soil)		
Super -P						
Uninoculated	5.1	5.3	5.2	22.0	22.1	22.1
(Rh)	7.9	7.2	7.6	22.8	22.1	22.5
(VAM)	10.5	10.3	10.4	50.1	48.3	49.2
Rh + VAM	15.8	15.2	15.5	64.3	62.3	63.4
Mean	9.8	9.5		39.8	38.7	
None nod.	4.2	4.1		21.0	22.0	
Rock-P						
Uninoculated	8.1	7.9	8.0	20.5	19.3	19.9
Rh	10.2	10.0	10.1	20.3	19.1	19.7
VAM	14.9	14.2	14.6	63.7	61.2	62.5
Rh + VAM	18.3	18.0	18.1	70.5	68.8	69.7
Mean	12.9	12.5		43.8	42.1	
Mone nod.	7.6	7.1		19.9	19.10	

	P Sources	N rate	I	PxI	NxI	PxNxI
Spore No.	1.21	N.S	1.97	2.01	2.01	2.10
Nodules weight	2.27	4.23	4.26	8.74	6.91	7.11

TABLE 3 : EFFECT OF N-RATE APPLICATION ON N AND P UPTAKE BY SOY BEAN PLANTS AS AFFECTED BY P SOURCES AND RHIZOBIUM AND/OR MYCORRHIZAE.

Treatments	N-uptake (mg/pot)			P-uptake (mg/pot)		
	20	100	Mean	20	100	Mean
	(mg N kg^{-1} soil)			*(mg N kg^{-1} soil)*		
Super -P						
Uninoculated	794.96	856.57	825.8	114.75	118.33	116.5
(Rh)	1188.80	1077.35	1133.1	126.08	122.92	124.5
(VAM)	495.67	1039.41	1017.5	153.39	149.58	151.5
Rh + VAM	1339.29	1246.35	1292.8	199.84	172.69	186.3
Mean	1079.7	1055.0		148.52	140.88	
None nod.	858.8	869.39		110.71	123.09	
Rock-P						
Uninoculated	632.94	784.33	708.6	105.49	108.55	107.02
Rh	998.15	886.45	942.3	119.02	132.88	125.9
VAM	845.83	811.13	828.5	142.48	137.29	139.9
Rh + VAM	1213.0	1176.42	1194.7	176.50	202.55	189.5
Mean	922.48	914.58		135.87	145.32	
Mone nod.	770.2	794.21		122.86	120.86	

	N. rate	P. sources	I	PxNxI
N uptake	120.34	65.47	133.11	171.37
P uptake	19.39	22.61	18.43	25.01

TABLE 4 : EFFECT OF N RATE APPLICATION ON ^{15}N DERIVED FROM FERTILIZER (Ndff), FROM ATMOSPHERE (Ndfa) AND N_2-FIXED BY SOY BEAN PLANTS AS AFFECTED BY P SOURCES, RHIZOBIUM AND/OR MYCORRHIZAE.

Treatments	Ndff	Ndfa	N2-fixed	Ndff	Ndfa	N2-fixed
	----- % ------		(mg/pot)	----- % ------		(mg/pot)
	(mg N kg^{-1} soil)			*(mg N kg^{-1} soil)*		
Super -P						
Uninoculated	20.4	2.90	23.05	56.65	0.58	4.99
Rh	11.0	47.64	566.34	46.90	17.56	189.18
VAM	18.8	10.52	104.74	55.56	2.07	21.52
Rh + VAM	10.2	51.45	689.07	42.83	24.96	311.09
None nod.	57.1	–	–	57.08	–	–
Rock-P						
Uninoculated	20.0	1.08	6.84	55.75	0.33	2.59
Rh	10.8	47.11	470.23	45.42	19.18	170.02
VAM	17.8	12.83	108.52	54.89	2.33	18.90
Rh + VAM	9.6	52.98	642.65	42.08	25.12	295.52
Mone nod.	56.2	–		56.20	–	–