RELATIONSHIP BETWEEN NITROGEN SOURCES, GERMINATION AND VIGOR OF SOYBEAN SEEDS[1]

J. MARCOS-FILHO[2]; C.C. CUSTODIO; G.M.S. CAMARA; E.A. MARCOS; R.E. MIRANDA
Departamento de Agricultura, ESALQ/USP, C.P. 9 - CEP: 13.418-900 - Piracicaba,SP

ABSTRACT: This research was conducted in two experimental areas; the first had been under cultivation with *Bradyrhizobium japonicum* anually inoculated for several years and the second was cultivated with sugarcane for many years receiving only mineral fertilization. Mineral nitrogen (urea and ammonium) effect was compared with the effects of inoculant treatments (liquid and peat carriers) on plant development, yield and seed quality. After harvesting, seeds were stored under normal environmental conditions and tested periodically. Germination, accelerated aging, electrical conductivity and seedling emergence tests showed that seeds coming from the area where inoculation was practiced have better quality.Best plant performance regarding plant height at different growth stages and yield was also found in this area. Among the nitrogen sources studied, best results were obtained when peat was the inoculant carrier.
Key Words: *Glycine max*, seeds, inoculation, germination, vigor.

RELAÇÕES ENTRE FONTES DE NITROGÊNIO, GERMINAÇÃO E VIGOR DE SEMENTES DE SOJA

RESUMO: A pesquisa foi conduzida em duas áreas experimentais do Departamento de Agricultura, ESALQ/USP. A primeira, cultivada com soja anualmente inoculada com *Bradyrhizobium japonicum*, durante vários anos e, a segunda, anteriormente cultivada com cana-de-açúcar, recebendo apenas adubação mineral. Os efeitos da aplicação de ureia e de sulfato de amônio foram comparados com os da inoculação de sementes, utilizando-se, produtos veiculados em turfa ou em meio líquido; avaliaram-se o desenvolvimento das plantas, a produção final e a qualidade das sementes (testes de germinação e de vigor) logo após a colheita e durante o armazenamento. Os resultados permitiram constatar o melhor desempenho fisiológico das sementes produzidas em área anteriormente cultivada com soja, o mesmo ocorrendo com o desenvolvimento inicial das plantas. Dentre as fontes de nitrogênio avaliadas, a utilização de inoculante veiculado em turfa se mostrou mais favorável.
Descritores: *Glycine max*, sementes, inoculação, germinação, vigor.

INTRODUCTION

Soybean seed inoculation with *Bradyrhizobium japonicum* has been a routine practice in different producing regions of Brazil. Thus, in addition to the amounts of nitrogen present in the soil, symbiotic fixation contributes significantly to insure the supply of this element, which is necessary for adequate plant development and production. Although the beneficial effects of inoculation are irrefutable, soybean response to the addition of nitrogen fertilizer to the soil and the effects of this procedure are contraditory (GIBSON, 1976; VARGAS, et al; 1982; FLORES *et al.*, 1987).

Several soybean growers and researchers have observed favorable as well unfavorable effects of the application of mineral nitrogen, depending upon the cultivar, quantity and source of nitrogen, sowing date and plant population/area, as well as environmental conditions.

The efficiency of the seed inoculation technique through *Bradyrhizobium* strains in peat carrier products is recognized in literature. However, even though promising, the information on the effects of the utilization of liquid carriers for the inoculant is less available, as pointed out by HAMMOUD (1981) and DANSO *et al.* (1990), among others.

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[2] Bolsista do CNPq.

Soybean response to the application of different sources of nitrogen has been evaluated through parameters such as number and weight of nodules per plant, development of root system, plant height, foliar area, number of pods per plant, yield and weight of seeds. Pratically no references were found on the effects upon physiological quality of the seeds produced; only SMITH & ELLIS (1980) suggested that soybean nodulation may be influenced by seedling vigor.

It should be pointed out, however, that relationships between nitrogen nutrition, the accumulation of proteins in the seed of other species and its possible influence on physiological quality were observed in studies carried out by CHING & RYND (1978), BULISANI & WARNER (1980), HADAVIZADEH & GEORGE (1988). It is well known that proteins are fundamental components of cell membrane system, whose integrity determines its selective permeability and is directly related with seed performance (BEWLEY & BLACK, 1985); these authors emphasized the importance of protein reserves for the normal seed metabolic activities by associating them with nucleic acid synthesis, formation of new tissues of the embryonic axis and enzyme activity.

In view of these considerations, the objective of this work was to study the relationships between nitrogen sources, inoculation method, yield and physiological quality of soybean seeds.

MATERIAL AND METHODS

The research was carried out in two experimental areas, located at Piracicaba, SP, Brazil (22°42'30" S; 47° 38' 00" W; 580m altitude).

Area A was under soybean cultivation during 1987/88, 1988/89 and 1989/90, seed having been inoculated annually with *Bradyrhizobium japonicum*; **Area B** had previously been under sugarcane cultivation.

Sowing was done in December 1990, utilizing cultivar IAC-8. A randomized block design with seven treatments, plus control, and five replications, separately for each experimental area, was adopted. The treatments, representing different nitrogen sources, consisted of mineral fertilizers in the form of ammonium sulphate (AS) and urea (U), inoculants carried in mineral oil (MO) or vegetal oil (VO), inoculants carried in peat (P_1 and P_2) and in emulsion (EI).

The plots corresponding to the mineral nitrogen treatments received 10kg/ha of N at sowing and 50kg/ha of N during the pre-flowering stage (V12). The inoculation with the products carried in peat was done at the rates of 250g and 1000g of inoculant per 100kg seeds, for areas A and B, respectively. For oily inoculants,80 and 240ml of the commercial product/100kg of seeds were utilized in areas A and B, respectively.

Each plot consisted of eight rows, spacing of 60cm row width and 5.0m row-lenght. All agricultural practices required for an adequate development of the plants, such as herbicide and insecticide applications, phosphate and potassium fertilization and, eventually, irrigation, were used in the experimental areas from the presowing phase on. Determinations consisted of initial stand, plant height at 21 days after seedling emergence and in the stages corresponding to R1/R2, R5 and R7 (FEHR & CAVINESS, 1977). After harvesting, the pods were manually shelled, the yield (kg/ha) and the weight of 1,000 seeds were evaluated, with values corrected for 13% moisture content. Next, the seeds were stored under room conditions during eight months.

Determinations of moisture content (oven method, 105°C for 24 hours), germination and vigor of seeds, were carried out after harvest and every four months. Tests of germination, electric conductivity and accelerated aging were performed according to the International Seed Testing Association Rules (1976) and the Seed Vigor Testing Handbook (ASSOCIATION OF OFFICIAL SEED ANALYSTS, 1983), respectively. Seedling emergence was evaluated 14 days after sowing two samples of 100 seeds for each replication, when seedlings reached the VC stage (FEHR & CAVINESS, 1977).

RESULTS AND DISCUSSION

TABLE 1 shows that the initial stand did not present marked variations,considering the effects of the treatments as well as those of experimental areas. The emergence reduction from seeds with emulsion inoculant (EI), even though statistically significant, is not considered sufficient to affect yield,due to the compensation ability presented by soybean plants.

TABLE 1. Stand (pl/m), yield (kg/ha) and weight 1000 seeds (g) as influenced by nitrogen sources and history of the area.

Treatment	Stand (pl/m)			Yield (kg/ha)			Weight 100 seeds (g)		
	A[1]	B	Mean	A	B	Mean	A	B	Mean
Control	26	26	26(*)	3351 Aa	1828 Bb	2589	169 Aa	157 BCb	163
U	24	25	25 AB	3009 Aa	2066 ABb	2537	174 Aa	149 Cb	162
AS	24	24	24 AB	2813 Aa	2159 ABb	2486	173 Aa	150 Cb	162
P_1	25	24	25 AB	3000 Aa	2507 Ab	2753	175 Aa	174 Aa	175
P_2	25	24	25 AB	2738 Aa	2505 Aa	2622	172 Aa	170 ABa	171
MO	25	25	25 AB	2762 Aa	1847 Bb	2305	172 Aa	151 Cb	162
VO	27	26	27 AB	3100 Aa	1913 ABb	2506	179 Aa	122 Cb	166
EI	23	23	23 B	3062 Aa	1733 Bb	2398	177 Aa	151 Cb	164
Mean	26a	26a		2979	2069		174	157	
C.V. (%)	5,0			12.7			4.7		

(*) Means within a column or within a row followed by the same capital or small letter, respectively, are not significantly different at P=0.05, according to Tukey test.

(1) A - area annually cultivated with soybeans
B - area previously cultivated with sugarcane

TABLE 2. Plant height (cm) at different developmental stages as influenced by nitrogen sources and history of the area.

Treatment	Plant height at 21 DAS[1] (cm)			Plant height at R1/R2 (cm)			Plant height at R5 (cm)			Plant height at R7 (cm)		
	A[(1)]	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	20.3	29.4	19.8(*)	94.5	91.4	93.5A	106.0	112.5	109.2 A	118.6	121.7	120.2 A
U	21.1	20.3	20.7 A	104.2	93.3	98.7A	130.5	114.7	122.6 A	127.6	125.4	126.5 A
AS	20.6	20.3	20.5 A	97.4	98.8	98.1A	119.5	113.5	116.5 A	121.2	127.8	124.5 A
P_1	20.1	21.6	20.9 A	105.8	87.7	96.8A	126.0	112.4	119.2 A	124.9	120.3	122.6 A
P_2	20.9	20.1	20.5 A	99.0	93.7	96.4A	123.7	110.3	117.0 A	126.0	120.5	123.2 A
MO	20.5	29.1	19.8 A	98.5	91.4	94.9A	127.3	115.8	121.5 A	119.5	112.8	116.2 A
VO	21.3	20.9	21.1 A	103.0	93.1	98.1A	128.9	117.4	123.2 A	127.7	122.5	125.1 A
EI	21.3	20.2	20.8 A	100.5	90.8	95.7A	122.5	116.2	119.3 A	127.8	119.1	123.4 A
Mean	20.8a	20.2a		100.4a	92.5b		123.0a	114.1b		124.2a	121.3a	
C.V. (%)	8.9			12,5			12.5			9.6		

(*) Means within a column or within a row followed by the same capital or small letter, respectively, are not significantly different at P=0.05, according to Tukey test.

(1) Days after sowing.

(2) A - area annually cultivated with soybeans

B - area previously cultivated with sugarcane

Thus, even in the absence of stand differences, significant effects of treatments were observed on the yield obtained in area B, which had not previously been cultivated with soybeans. High performance was noted with the utilization of inoculants carried in peat (P_1 and P_2), while emulsion inoculant (EI), the one carried in mineral oil (MO) and control exhibited lower performance.

It was also shown that soybean yield in area A was significantly higher than in area **B**, for all treatments, except for P_2. This superiority was also revealed in the results obtained for weight of 1000 seeds, where, once again, the high performance of treaments P_1 and P_2 was evident.

Therefore, it was observed that annual inoculation presents advantages as compared with cultivation in areas where, in previous years, the crop utilized had received only mineral nutrition; similarly, in this situation, the response to the inoculant carried in peat was shown to be advantageous.

On the other hand, plant height as determined during different stages of development (TABLE 2) showed only marked variations in R1/R2 and in R5, when, although there were no treatment effects, the growth of plants cultivated in area A was greater. The stages mentioned comprise the period of the greatest photosynthesis activity of soybeans and therefore, these results reinforce advantages of growing soybeans in areas that annually receive inoculated seeds, thus insuring the presence of an adequate concentration of *Bradyrhizobium japonicum*, in addition to the unquestionable benefits of organic matter. Plant height, dry weight of shoot and roots as well as number of pods/plant of the plants grown in area A were higher than those of area **B** (data not presented).

Seed moisture content was shown to be favorable for conservation during the whole storage period, although only data obtained during the testing times are presented in TABLE 3. TABLE 4 shows the data of seed physiological quality, soon after harvest. In general, there were no significant variations between treatments and between areas in germination, accelerated aging and emergence tests, even though the treatments with inoculants carried in peat (P_1 and P_2) tended to show a better performance, mainly in area B.

This same tendence remained at four months of storage (TABLE 5), although seed moisture content differences should have contributed for an over estimation of seed performance through the conductivity test for area A (higher moisture content) and through the accelerated aging test for area B (lower M.C.). At eight months of storage, there was no significant decline in germination, as compared with the former period, but the emergence of seedlings was seriously affected by the unfavorable environment (excessive soil moisture). In both tests, the treatments did not differ one from another, however the seeds produced in **area A** show better performance than those produced in **area B**. In the accelerated aging test the better quality of the seeds of **area A** and the influence of the treatments on the performance of the seeds of **area B** were confirmed, but the lower values observed for these seeds in the conductivity test, compared with those obtained after four months of storage, remain unexpected.

The results of the electrical conductivity test also showed the consistent superiority of the physiological quality of the seeds produced in **area A**. They also showed the higher performance of the treatments with inoculants carried in peat, in **area B**, together with the results of yield, weight 1,000 seeds and plant height in R1/R2 and in R5.

The electrical conductivity test indirectly evaluates seed vigor, reflecting the integrity of the cell membrane system. This test is considered sensitive to relatively small variations in seed vigor and this characteristic was once again verified in this study, when the conductivity results were compared with those of the germination and accelerated aging.

The importance of the protein reserves in seeds, their relationship with the membrane system and metabolic activities were verified by previously mentioned researchers. Therefore it is reasonable to suppose that the utilization of inoculants carried in peat would have favored the development of plants and the production of seeds with higher protein content as compared with the other treatments, specially in soil not previously cultivated with soybeans. This possibility was confirmed by REGITANO-d'ARCE *et al.* (1992). Chemically analysing the same materials utilized in the present study,these workers found that peat carrier inoculants conducted to the highest protein content seeds, as well as the best quality oil regarding free fatty acids and peroxide values.

Therefore, the results here presented clearly indicate the advantages,in terms of yield and physiological quality, of soybean seed production in soils with a history of inoculation.

TABLE 3. Seed moisture content (%) after harvest and after four and eight months of storage.

Treatment	After harvesting			Storage 4 months			Storage 8 months		
	A(1)	B	Mean	A	B	Mean	A	B	Mean
Control	12.1	13.9	13.0	9.9	10.0	10.0	12.3	12.0	12.2
U	12.0	13.6	12.8	10.3	10.0	10.2	12.4	12.1	12.3
AS	13.1	13.5	12.3	10.1	10.4	10.3	12.2	12.1	12.2
P_1	12.0	13.3	12.7	9.8	10.1	10.0	12.3	12.3	12.3
P_2	12.5	12.8	12.7	10.0	11.0	10.5	12.0	12.3	12.2
MO	12.0	12.7	12.4	9.9	10.0	10.0	12.3	12.3	12.3
VO	12.2	13.0	12.6	10.0	10.1	10.1	12.3	12.2	12.3
EI	12.4	14.0	13.2	9.9	10.0	10.0	12.5	12.2	12.4
Mean	12.3	13.4		10.0	10.2		12.3	12.2	

(1) A - area annually cultivated with soybeans
B - area previously cultivated with sugarcane

TABLE 4. Seed germination and vigor after harvesting, as influenced by nitrogen sources and history of the area.

Treatment	Germination (%)			Conductivity (umhos/g/cm)			Acc. Aging (%)			Seedling emergence (%)		
	A[1]	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	91	90	91A(*)	40.3	58.5	49.4 ABC	86	87	87 A	95	92	94
U	92	90	91 A	42.4	59.3	50.9 ABC	86	88	87 A	91	97	89
AS	91	88	90 A	41.2	60.8	51.0 ABC	88	85	87 A	91	91	91
P_1	90	94	92 A	44.0	50.3	47.1 AB	85	93	89 A	91	97	94
P_2	92	95	94 A	43.0	48.6	45.8 A	87	93	90 A	93	96	95
MO	89	92	91 A	45.2	56.9	51.1 ABC	82	91	87 A	97	92	90
VO	93	89	91 A	43.4	62.1	52.8 BC	86	89	88 A	95	91	93
EI	93	91	92 A	45.7	61.6	53.6 C	84	88	86 A	93	91	92
Mean	91a	91a		43,1a	57.3b		86b	89a		92a	92a	
C.V. (%)	6.6			8.3			7.3			7.4		

(*) Means within a column or within a row followed by the same capital or small letter, respectively, are not significantly different at P=0.05, according to Tukey test.

(1) A - area annually cultivated with soybeans
B - area previously cultivated with sugarcane

TABLE 5 - Seed germination and vigor after four months of storage as influenced by nitrogen sources and history of the area.

Treatment	Germination (%)			Conductivity (umhos/g/cm)			Acc. Aging (%)			Seedling emergence (%)		
	A[1]	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	92 Aa(*)	94 ABa	93	51.0 Aa	76.5 BCb	63.8	86	93	90 A	89	72	82 A
U	91 Aa	92 ABa	92	51.1 Aa	77.9 BCb	64.5	79	91	85 A	82	76	79 A
AS	91 Aa	91 ABa	91	54.7 Aa	79.0 Bcb	66.9	86	89	88 A	87	71	79 A
P_1	89 Ab	97 Aa	93	53.8 Aa	67.8 ABb	60.8	79	95	87 A	85	80	83 A
P_2	93 Aa	96 ABa	95	53.3 Aa	64.2 Ab	58.8	81	96	89 A	87	73	80 A
MO	91 Aa	94 ABa	93	52.1 Aa	74.0 ABCb	63.1	75	92	84 A	82	74	78 A
VO	91 Aa	90 Ba	91	52.3 Aa	80.2 Cb	66.3	86	91	89 A	84	73	79 A
EI	95 Aa	89 Ba	92	52.8 Aa	80.9 Cb	66.9	81	90	86 A	85	62	74 A
Mean	92	93		52.6	75.1		82b	92a		85a	73b	
C.V. (%)	5.8			9.2			7.3			9.1		

(*) Means within a column or within a row followed by the same capital or small letter, respectively, are not significantly different at P=0.05, according to Tukey test.

(1) A - area annually cultivated with soybeans
B - area previously cultivated with sugarcane

TABLE 6 - Seed germination and vigor after eight months of storage as influenced by nitrogen sources and history of the area.

Treatment	Germination (%)			Conductivity (umhos/g/cm)			Acc. Aging (%)			Seedling emergence (%)		
	A[1]	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	93	88	91 A(*)	49.0 Aa	60.3 ABB	54.7	91 Aa	64 BCb	78	68	40	54 A
U	91	90	91 A	52.4 Aa	61.3 ABb	56.9	86 Aa	60 Cb	73	61	53	57 A
AS	91	83	87 A	53.4 Aa	59.7 ABa	56.6	83 Aa	55 Db	69	61	44	53 A
P_1	90	89	90 A	53.1 Aa	53.6 Aa	53.4	82 Aa	85 Aa	84	64	47	56 A
P_2	94	90	92 A	54.2 Aa	50.0 Aa	52.1	84 Aa	83 Aa	84	66	52	59 A
MO	89	88	89 A	52.2 Aa	61.4 ABb	56.8	84 Aa	56 Db	70	63	53	58 A
VO	92	84	88 A	52.2 Aa	65.9 Bb	59.1	83 Aa	58 CDb	71	69	40	50 A
EI	95	86	91 A	50.3 Aa	66.2 Bb	58.3	88 Aa	48 Db	68	67	50	59 A
Mean	92a	87b		52.1	59.8		85	64		64a	47b	
C.V. (%)	8.3			10.5			11.1			18.4		

(*) Means within a column or within a row followed by the same capital or small letter, respectively, are not significantly different at P=0.05, according to Tukey test.

(1) A - area annually cultivated with soybeans
B - area previously cultivated with sugarcane

Although the effects of different nitrogen sources have been observed mainly in the area not previously cultivated with soybeans, there were evidences of a higher efficiency of peat as an inoculant carrier and of problems with the utilization of emulsion inoculant.

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