EFFECT OF SEED TREATMENTS AND TIME OF INOCULATION ON NODULATION AND YIELD OF SOYBEANS[1]

S.A.Z. MAHMOUD[2], M. ABDEL-NASSER[3] and F.S. ALI[4]

ABSTRACT - Two field experiments were run using various seed treatments in attempt to obtain successful nodulation and yield of 'Harosoy' soybean (*Glycine max* (L.) Merrill). Soaking 'Harosoy' soybean seeds in the inoculum *R. japonicum* E 45 for three hours or germinating the seeds (by washing with tap-water for five minutes every one hour and removal of excess water from seed-container) for 3, 6, 12 or 24 hours (12 hrs-treatment was the best) and inoculation with *R. japonicum* E 45 just before sowing resulted in obtaining successful nodulation and yield. This was indicated by the formation of appreciable high number of nodules on 45, 55, 65, 75 or 85th days after sowing. On harvest, dry weight of the resulting seeds and of whole yield and total-N content of seeds and whole yield as well as dry weight of 100 seeds were substantially higher as compared with control non-inoculated treatments (or even inoculated after 10, 15 or 20 days after sowing).

Index terms: (*Glycine max* (L.) Merrill), *Rhizobium japonicum*, sowing, N-content of seeds, non inoculated.

EFEITO DO TRATAMENTO DE SEMENTES E DE TEMPO DE INOCULAÇÃO, NA NODULAÇÃO E PRODUÇÃO DE SOJA

RESUMO - Dois experimentos a campo, com vários tipos de tratamento de sementes de soja (*Glycine max* (L.) Merrill), visando obter boa nodulação e produção de soja 'Harosoy'. O embebimento de sementes de soja "Harosoy" no inóculo *R. japonicum* E 45 durante três horas, ou a germinação de sementes (lavadas durante cinco minutos a cada hora, durante 3, 6, 12 ou 24 horas sendo o tratamento de 12 horas o melhor) e a inoculação com *R. japonicum* E 45 imediatamente antes da semeação trouxeram como resultado nodulação e produção bem sucedidas. É o que indica a formação de números bastante elevados de nódulos no 45º, 55º, 65º, 75º e 85º dias após a semeadura. Na colheita, o peso das sementes daí resultantes, bem como a produção total e peso seco de 100 sementes foram substancialmente maiores, em comparação com as testemunhas não-inoculadas (ou mesmo inoculadas, porém somente 10, 15 ou 20 dias após a semeadura).

Termos para indexação: *Glycine max* (L.) Merrill), *Rhizobium japonicum*, semeadura, conteúdo de N nas sementes, não-inoculado.

INTRODUCTION

Several authors had referred to the presence of inhibitiory substances (to specific Rhizobia) in seeds of soybeans as well as in other leguminous plants (Fawaz et al. 1970, Iswaran 1970, Dadarwal & Sen 1971 and 1973, El-Mallah 1974, Jain & Rewari 1976, and Abdel-Ghaffar 1977). Very recently, Abdel-Nasser et al. (1979 a, b) could detect antibacterial substances present in the seeds (cotyledon, dicotyledon, and seed-coat) of 'Harosoy' soybean cultivar which, on laboratory medium, inhibited the growth of *Rhizobium japonicum* E 45. The antibacterial substances were water soluble or partially insoluble and could be eliminated, on laboratory medium, by certain chemical treatments as well as by germination for 12 hours before inoculation. On such basis, the present field experiments were carried out to obtain successful nodulation by treating seeds before inoculation to remove the harmfullness of antibacterial substances.

MATERIALS AND METHODS

Two field experiments were carried out (March 1977) at the experimental farm of the Faculty of Agriculture, Minia, Egypt, for studying: (1). Effect of inoculation with the efficient strain of *Rhizobium japonicum* E 45 (Abdel-Nasser et al. 1979 a) either before planting (soaking seeds in the inoculum for 3 hrs) or after 10, 15 or 20 days after sowing (the broth cultures of *R. japonicum* were diluted with water and added to the soil around the base of the stem) on nodulation and yield of 'Harosoy' soybeans, and (2). Effect of seed germination (washed with tap-water for five minutes every one hour during 3, 6, 12 or 24 hrs and removal of excess water from the seed-container) prior to inoculation with *R. japonicum* E 45 on nodulation and yield of 'Harosoy'

[1] Aceito para publicação em 24 de novembro de 1980.

[2] Ph.D., Prof. Dr.; Faculty of Agriculture, Ain Shams Univ. Shoubra El Khema, Cairo, Egypt.

[3] Ph. D., Prof. Dr., Faculty of Agriculture, Minia Univ., Minia, Egypt.

[4] M.Sc., Faculty of Agriculture, Minia Univ., Minia, Egypt.

soybeans. Each experiment was carried out in complete randomized blocks design using five seed treatments (including non-inoculated seeds as control) in four replicates; each plot was $\frac{1}{200}$ Feddan (seven rows). Feddan = 4.200 m^2). The first irrigation was after two weeks from planting and then at 15-20 days intervals. After 45, 55, 65, 75 or 85 days from planting, ten plants from a row of each plot were carefully uprooted and gently washed for counting the nodules formed on each plant. After harvest, the dry weight of seed yield (as well as of 100 seeds) and of the whole plant (seeds, pods, shells, shoots and roots) were determined. Total nitrogen contents of the seeds and of the whole plants were determined as previously mentioned (Mahmoud et al. 1978 and Abdel-Nasser et al. 1979a). The obtained data were statistically analyzed.

The used seeds of 'Harosoy' soybeans were those resulting in the year 1976 from the previous experiment (Mahmoud et al. 1978) and originally obtained from legume Crops Section, Agriculture Research Center, Giza, Egypt. The soil (Clay loam with pH 8.1, total-N 0.165% and solube phosphorus 2.29 mg/100 g soil) was amended with 200 kg superphosphate/Fed. before planting.

Rhizobium. japonicum strain E 45 (from INTA Argentina, 1967) which has been selected in the previous experiments (Abdel-Nasser et al. 1979a) was inoculated in flasks containing 1000 ml of sterilized yeast extract mannitol broth "Medium 79" (Allen 1959) and incubated at 28°C for six days prior to use.

RESULTS AND DISCUSSION

Results obtained from the previous experiment (Abdel-Nasser et al. 1979b) indicated the presence of antibacterial substances in seeds of 'Harosoy' soybean cultivar which, on laboratory medium, inhibited the growth of *Rhizobium japonicum* E 45. However, the toxic substances were found to be water soluble or partially insoluble. On such basis, two field experiments were carried out in this investigation using different seed treatments in attempts to eliminate (or decrease) the toxic effects of these substances in order to obtain, under field conditions, successful nodulation and yield of 'Harosoy' soybean. The results obtained from both experiments are presented in Tables 1 and 2 and generally indicate the failure of non-inoculated seeds to form nodules all over the experiment. This could be attributed mainly to absence of adequate densities of effective soybean-rhizobia strain in used soil, and partially to the presence of antibacterial substances in seeds (Abdel-Nasser et al. 1979 a, b). Similar findings, however, had been reported on soybean as well as on other leguminous plants growing in different localities at different countries (Fawaz et al. 1970, Iswaran 1970, Dadarwal & Sen 1971 and 1973, El Mallah 1974, Jain & Rewari 1976 and Abdel-Ghaffar 1977).

In the first experiment (Table 1), inoculation was carried out either before or after sowing. Inoculated soaked seeds resulted in the formation of the highest numbers of nodules on 45, 55, 65 or 75th day after sowing. On the other hand, although few number of nodules were found on plants inoculated after 10, 15 or 20 days after sowing, the determined dry weight or total-N content of the resulting seeds and of whole yield as well as dry weight of 100 seeds were however nearly similar compared with non-inoculated treatment. Therefore, it could be concluded that soaking 'Harosoy' soybean seeds in the inoculum of *R. japonicum* E 45 for three hrs just before sowing resulted in successful nodulation and yield whereas inoculation after 10, 15 or 20 days after sowing showed no response.

In the second experiment, inoculation was carried out immediately after germinating the seeds. The results presented in (Table 2) generally indicated that germinated-inoculated seed treatments formed appreciable high number of nodules on 45, 55, 65, 75 or 85th day after sowing. On harvest, the increases recorded for dry weight of seeds (kg/4.200 m^2) obtained from all treatments as well as dry weight of 100 seeds were substantially higher as compared with control. The increase recorded for total-N content of the seeds and of the whole yield were either highly significant (for seeds germinated for 3,6 or 12 hrs) or significant (for those germinated for 24 hrs). Generally, the germinated (for 12 hrs) inoculated treatment seemed to be the best one as it resulted in obtaining the highest yield of seeds and of whole yield with highest total-N content and dry weight of 100 seeds. This is in harmony with Abdel-Nasser et al. (1979 b), who could eliminate, on laboratory medium, the toxic effect of the antibacterial substances detected in seeds of 'Harosoy' soybean on *Rhizobium japonicum* E 45 by germinating the seeds for 12 hrs.

Generally, it is recommended for improvement of nodulation and yield of 'Harosoy' soybean to soak the seeds in the inoculum for three hrs or germinating the seeds for 3-12 hrs (12 hrs was the best treatment), then inoculating just before sowing. Inoculation with an efficient strain of *Rhizobium japonicum* such as with the foreign one E 45 (Abdel-Nasser et al. 1979 a) seemed to be necessary. The formed nodules were, mostly, reddish or pink in colour, big in size, round in shape and were mainly formed on the main root and nearby on the secondary roots (Fig. 1). These are characteristics of effective nodules (Mahmoud et al.

TABLE 1. Effect of inoculation with *R. japonicum* E45 either before sowing (by soaking the seeds in the inoculum for 3 hrs) or after 10, 15 or 20 days after sowing on nodulation and yield of 'Harosoy' soybeans.

Seed treatments		Numbers of nodules/10 plants				Dry weight (kg/4.200m²)		Total - N (kg/4.200m²)		Dry weight of 100 seeds (g)
		Days after sowing				Seeds	Total yield	Seeds	Total yield	
		45	55	65	75					
Non-inoculated (control)		0.0	0.0	0.0	0.0	567 5	1846.2	35.52	53.84	10.96
Inoculated	Soaked for 3 hrs (in bacterial susp.)	152.3	199.3	212.8	97.3	840.2	2250.5	53.43	80.63	13.28
	After 10 days from sowing	0.0	79.5	41.0	51.0	596.7	1697.5	37.44	54.94	11.27
	After 15 days	0.0	15.0	41.5	21.0	554.7	1723.7	35.09	54.96	10.97
	After 20 days	0.0	0.0	4.7	9.0	533.2	1601.2	34.63	52.91	10.84
L.S.D.	0.05	-	77.5	71.0	52.4	89.8	168.0	7.67	6.17	0.6738
	0.01	-	117.5	102.0	75.3	125.9	235.5	10.76	8.65	0.9447

TABLE 2. Effect of inoculation with *R. japonicum* E45 just after germinating the seeds (by washing with tap water for 5 minutes every one hr and removal of excess water) for 3, 6, 12 or 24 hrs on nodulation and yield of 'Harosoy' soybeans.

Seed-treatments		Numbers of nodules/10 plants				Dry weight(kg/4.200m²)		Total-N (kg/4.200m²)		Dry weight of 100 seeds (g)
		Days after sowing				Seeds	Total yield	Seeds	Total yield	
		45	55	65	75	85				
Non-inoculated (control)		0.0	0.0	0.0	0.0	0.0	590.7	2275.1	37.25	64.29	11.30
Inoculated germinated seeds (washed)	3 hrs	95.5	222.5	249.0	334.5	227.3	850.0	2563.4	54.04	84.67	13.45
	6 hrs	115.8	139.0	182.8	261.3	199.3	885.5	2730.0	55.19	90.68	13.77
	12 hrs	115.8	208.5	237.0	218.5	209.8	906.5	2905.9	58.31	98.91	14.02
	24 hrs	129.3	163.3	216.3	241.8	154.3	782.2	2335.2	49.76	80.16	13.61
L.S.D.	0.05	NSD	NSD	NSD	NSD	NSD	119.84	NSD	9.46	12.25	0.46
	0.01	NSD	NSD	NSD	NSD	NSD	168.02	NSD	13.46	18.18	1.42

1970, Alexander 1977 and Abdel-Nasser et al. 1979a). It may remain worthy to mention that gentle treatment of seeds as described above did not affect seed germination. However, the yield of soybean during that year was in general low.

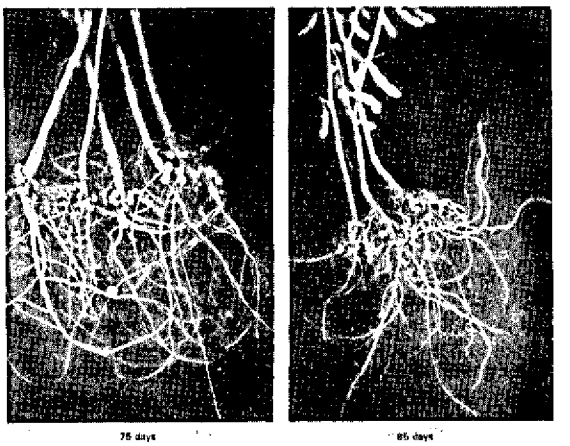

FIG. 1. Nodulation of germinated (12 hrs.) inoculated seeds of 'Harosoy' soybean on different periods of growth.

REFERENCES

ABDEL-GHAFFAR, S.A.M. **Some factors affecting-nodulation and nitrogen fixation of soybean** *(Glycine max).* Egypt. University of Alexandria, Fac. Agric., 1977. Tese Mestrado.

ABDEL-NASSER, M.; MAHMOUD, S.A.Z.; MAKAWI, A. & ALI, F.S. Studies on nodulation of soybean in Egypt. I. Selection of an efficient strain of *Rhizobium japonicum*. **Zbl. Bakt., 134**(2):666, 1979a.

ABDEL-NASSER, M.; MAHNOUD, S.A.Z.; MAKAWI, A. & ALI, F.S. Studies on nodulation of soybean in Egypt. II. Effect of seed-diffusates on *Rhizobium japonicum*. **Zbl. Bakt., 134**(2):600, 1979b.

ALEXANDER, M. **Introduction to soil microbiology.** 2.ed. New York, John Wiley and Sons, Inc. 1977.

ALLEN, O.N. **Experiments in soil bacteriology.** 3.ed. Minneapolis Burgess Publ. Co., 1959.

DADARWAL, K.R. & SEN, A.N. Inhibitory effect of seed diffusates of some legumes on rhizobia and other bacteria. **Indian J. Agric. Sci., 43**:82, 1973.

DADARWAL, K.R. & SEN, A.N. Survival of *R. japonicum on soybean seeds.* **Indian J. Agric. Sci., 41**:564, 1971.

EL-MALLAH, M.I. **Studies on soyabean.** Cairo, Egypt, Al-Azhar Univ., Fac. Agric., 1974. Tese Mestrado.

FAWAZ, K.M.; ABDEL-GHAFFAR, A.S. & EL-GABALY, M.M. Inhibition effect of bean seeds on Rhizobia. **Agrokemia ès Talajtan, 19**(4):546, 1970.

ISWARAN, V. Soaking of soybean seed and its effect on nodulation and yield. **Mysore J. Agric. Sci., 4**: 364, 1970.

JAIN, M.K. & REWARI, R.B. Studies on seed coat toxicity to rhizobia of urid (*Phaseolus mungo*), mung (*Phaseolus aureus*) and soybean (*Glycine max*). **Zbl. Bakt., 131**:193, 1976.

MAHMOUD, S.A.Z.; MAKAWI, A.; ABDEL-NASSER, M. & ALI, F.S. Studies on nodulation of soyabeans in Egypt. III. Inoculation and application of fertilizers. **Egypt. J. Microbiol., 13** (12):91, 1978.

MAHMOUD, S.A.Z.; TAHA, S.M. & SALEM, S.H. Relation between the efficiency of Rhizobia strains and the amino acid content in nodules and roots of some leguminous plants. **Agrokemia ès Talaitan.** 19(4):531. 1970.