

## Full Length Research Paper

# Nodulation and nitrogen fixation of field grown common bean (*Phaseolus vulgaris*) as influenced by fungicide seed treatment

Ndeye Fatou Diaw GUENE, Adama DIOUF and Mamadou GUEYE\*

MIRCEN/ Laboratoire commun de microbiologie IRD-ISRA-UCAD, BP 1386, DAKAR, Senegal

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A field experiment was conducted at Bel Air station, in Dakar using  $^{15}\text{N}$  isotope dilution technique and the non nodulating soybean (*Glycine max*) variety m129 as reference plant to test the compatibility of Dichlorofenthion-thiram (DCT) fungicide to the inoculation of common bean (*Phaseolus vulgaris*) Paulista variety with both *Rhizobium etli* ISRA 353 and *R. tropici* strain ISRA 554. Nodulation was not induced with *R. etli* ISRA 353 and nitrogen fixation did not occur. With *R. tropici* ISRA 554, a decrease in nodulation was observed, but nitrogen fixation was not significantly different compared to that of the non DCT-treated common bean.

**Key words:** Common bean, fungicides, isotope dilution,  $^{15}\text{N}$ , nitrogen fixation, nodulation, *Phaseolus vulgaris*, *Rhizobium*.

## INTRODUCTION

In Senegal, common bean (*Phaseolus vulgaris*) needs to be inoculated with elite *Rhizobium* strains in the growing area called Niayes zone (Diouf et al., 1999). Usually, seeds of common bean supplied to farmers are often treated with fungicide to prevent losses due to seed-borne pathogens.

Studies on compatibility of rhizobial strains with fungicides are currently controversial. Application of Captan, Pentachloronitrobenzene (Curley and Burton, 1975), and Apron (Rivellin et al., 1993), on soybean (*Glycine max*) seeds reduced the viability of *Bradyrhizobium japonicum* by 18, 75 and 61%, respectively, after 1 h exposure. Graham et al. (1980) observed that less than 10% of *R. phaseoli* strains survived on Thiram-treated seeds of common bean. By contrast, no detrimental effect was found on the compatibility of Apron with *R. japonicum* applied to

soybean seeds (Diatloff, 1986) or with *R. meliloti* on alfalfa seeds (Edmisten et al., 1988).

Since compatibility between rhizobial inoculants and fungicides has not been so far studied in Senegal, our objective was to examine effect of inoculation of fungicide-treated seeds of a common bean variety, Paulista which is one of the most commonly grown varieties in Senegal, in relationship to growth and grain yield, nodulation and nitrogen fixation.

## MATERIALS AND METHODS

A field experiment was carried out in Dakar, at Bel-Air experimental station. The soil was a sandy type (94% of sand) containing approximately 100 native *Rhizobium/g*, counted by infection test method (Brockwell, 1982 ; Vincent, 1970) using common bean seedlings. The pH of the soil was 7.0 with 0.025% nitrogen (Bremner, 1965), and 40 ppm available phosphorus (Olsen et al., 1954). The seeds of common bean variety, Paulista, and that of non nodulating soybean variety m129 used as non-fixing control plant were hand sown in a randomised completed bloc design with four replicates. The size of each plot was 1.35 x 2.55 m, with 15 cm and 45 cm within and between rows respectively. There were four treatments: (i) seeds of common bean treated with Dichlorofenthion-thiram(DCT) fungicide and inoculated with *R. etli*

\*Corresponding author; phone: +221-8493321, fax: +221-8321675; e-mail: Mamadou.Gueye@ird.sn

**Table 1.** Shoot (SDW) and nodule (Nod. DW) dry weights and pod yield of field grown common bean (*P. vulgaris*) Paulista variety cultivated at Bel Air experimental station, inoculated with *Rhizobium* strains ISRA 353 and ISRA 554 and treated with Dichlorofenthion-thiram (DCT) fungicide.

<i>Rhizobium</i> strains	Fungicide	SDW (Mg ha <sup>-1</sup> )	Nod. DW (mg pl. <sup>-1</sup> )	Pod yield (Mg ha <sup>-1</sup> )
ISRA 353	None	1.4 a	31.5 a	1.4 a
	DCT	1.1 a	0.0 b	0.0 a
CV (%)		20.4	16.9	21.2
ISRA 554	None	2.3 a	86.2 a	2.6 a
	DCT	2.6 a	59.2 b	2.2 a
CV (%)		20.7	18.0	22.0

In each column, for each *Rhizobium* strain, values followed by the same letter do not differ significant at p = 0.05

strain ISRA 353 (Diouf et al., 2000); (ii) seeds of common bean non treated with DCT and inoculated with *R. etli* strain ISRA 353; (iii) seeds of common bean treated with DCT and inoculated with *R. tropici* strain ISRA 554 (Diouf et al., 2000); (iv) seeds of common bean non-treated with DCT and inoculated with *R. tropici* strain ISRA 554.

Rhizobial inoculants were applied as peat slurry containing 10<sup>7</sup> *Rhizobium*/g. The rhizobial strains ISRA 353 and ISRA 554 were selected at MIRCEN rhizobial culture collection for their high nitrogen fixing potential in association with common bean (Guene, 2002). Within each plot, a 0.60 X 0.45 m microplot was delimited for the application of <sup>15</sup>N-labelled fertilizer solution, (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> containing 5 atom % <sup>15</sup>N excess to supply 20 kg N ha<sup>-1</sup>. Unlabelled (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was applied at the same rate to the remaining plots. To all plots a basal fertilizer was added and consisted of 60 kg P ha<sup>-1</sup> as a triple superphosphate and 120 kg K ha<sup>-1</sup> as KCl.

At 60 days after sowing, all plants were harvested from the microplots. The harvested plants were separated into different parts. Nitrogen content (%N) and atom % <sup>15</sup>N excess (%<sup>15</sup>Nae) for individual plant part were determined at the laboratory of soil biochemistry in ISRA-IRD centre of Dakar, Bel Air. Nitrogen fixation (%Ndfa) was estimated using the isotope dilution equation (Fried and Middelboe, 1977):

$$\%Ndfa = \left( 1 - \frac{\% \text{ } ^{15}\text{Nae in fixing crop}}{\% \text{ } ^{15}\text{Nae in non fixing crop}} \right)$$

Data were statistically analysed using the Newman and Keuls test.

## RESULTS

### Use of *Rhizobium* strain ISRA 353

That the seeds were treated or not with the DCT, there were a good vegetative development of the plants and a good production of pods. There was however no significant difference on shoot dry weight and pod yield between the plants from DCT-treated seeds and that

from non DCT-treated seeds. Averaged shoot dry weight and pod yield were 1.3 Mg ha<sup>-1</sup> and 1.2 Mg ha<sup>-1</sup> respectively (Table 1). On the other hand, no nodule was found on the roots of plants derived from DCT-treated seeds. Consequently nitrogen fixation did not occur in these plants, whereas average of 31.5 mg plant<sup>-1</sup> of dry nodules recorded on the plants derived from non DCT-treated seeds (Table 1) resulted in 18 and 35% as the proportion of nitrogen derived from fixation (%Ndfa) in the shoot and in the pods, respectively. Corresponding amounts of nitrogen derived from fixation (Ndfa) were 6 kg N ha<sup>-1</sup> and 15.4 kg N ha<sup>-1</sup> (Table 2).

### Use of *Rhizobium* strain ISRA 554

The plants were also well developed. No significant difference was observed on the shoot dry weight between plants from treated and non DCT-treated seeds with an average of 2.45 Mg/ha. The pod yield was however decreased (-19.7%) by treating the seeds with DCT (Table 1). Although nodulation of plants was not inhibited by the DCT, nodules dry weight was decreased in the plants from DCT-treated seeds in comparison to that of plants from non DCT-treated seeds, 59.2 mg plant<sup>-1</sup> vs 86.2 mg plant<sup>-1</sup> (Table 1). No difference was however observed in both the %Ndfa and Ndfa between the two treatments: 38.2% and 18.5 kg N ha<sup>-1</sup> respectively in the shoot, 54% and 48.9 kg N ha<sup>-1</sup> in the pods (Table 2).

## DISCUSSION

Organic fungicides are usually used in agriculture in order to protect seeds to diseases caused by fungi. The main known fungicides are however toxic to rhizobia (Diatloff,

**Table 2.** Nitrogen content (%N) and total nitrogen (Total N), atom %<sup>15</sup>N excess (%<sup>15</sup>Nae), proportion (%Ndfa) and amount (Ndfa) of nitrogen derived from atmosphere of field grown common bean (*P. vulgaris*) Paulista variety cultivated at Bel Air experimental station, inoculated with *Rhizobium* strains ISRA 353 and ISRA 554 and treated with Dichlorofenthion-thiram (DCT) fungicide.

<i>Rhizobium</i> strains	Plant organs	Fungicide	%N	Total N (kg ha <sup>-1</sup> )	% <sup>15</sup> Nae	%Ndfa	Ndfa (kg ha <sup>-1</sup> )
ISRA 353	Shoot	None	2.0 a	27.2 a	0.4 b	18.4	6.0
		DCT	2.3 a	24.3 a	0.6 a	0.0	0.0
	Pods	None	3.4 a	46.1 a	0.4 b	35.0	15.4
		DCT	3.4 a	34.0 b	0.7 a	0.0	0.0
CV (%)			18.2	20.3	18.7	21.4	24.1
ISRA 554	Shoot	None	1.8 a	43.3 a	0.3 a	37.9 a	16.2 a
		DCT	2.2 a	54.5 a	0.3 a	38.5 a	20.7 a
	Pods	None	3.6 a	95.1 a	0.2 b	58.3 a	56.4 a
		DCT	4.0 a	81.6 a	0.3 a	49.7 a	41.1 b
CV (%)			20.3	19.2	20.1	22.6	23.5

In each column, for each *Rhizobium* strain and for each plant organ, values followed by the same letter do not differ significant at  $p = 0.05$

1970; Hofer, 1958). In most cases, the rhizobia remain viable, but are not able to nodulate the host plants or their ability to fix nitrogen is reduced (Fisher, 1976; Staphorst and Strijdom, 1976). DCT is derived from thiram (tetramethyl-thiram-disulphide), one of the less toxic fungicide to rhizobia. We have tested the compatibility of DCT to the inoculation of Paulista variety with *Rhizobium* strains ISRA 353 and ISRA 554. In the first case there is incompatibility: no nodulation was induced and nitrogen fixation did not occur. In the second case, there is compatibility with less nodulation, and equivalent fixed nitrogen compared to that of plants from non DCT-treated seeds. In addition to the results of Hashem et al. (1997) indicating difference in compatibility with fungicides between peanut (*Arachis hypogaea*) and *Bradyrhizobium* inoculants, our results reflect and reactivate the discussion on inoculation of fungicides-treated seeds because the discrepancy between ISRA 353 and ISRA 554 rhizobial strains may be due to the difference of *Rhizobium* species, that is, *R. etli* and *R. tropici*. Considerable differences in tolerance among species and strains of rhizobia to different fungicides have been reported by Tesfai and Mallik (1986).

The effect of DCT on the nodulation of common bean Paulista variety inoculated with *Rhizobium* strain ISRA 353 was similar to that observed with *Cicer arietinum* (Thomas and Vyas, 1984; Welty et al., 1988), *Glycine max* (Teskai and Mallik, 1986) and Pigeon pea (Rennie et al., 1985) treated with different fungicides. Although effect of fungicide application on the viability of *Rhizobium*

strains has been already reported by several authors, the difference in nodulation of the Paulista variety by the *Rhizobium* strains ISRA 353 and ISRA 554 as influenced by DCT-treated seeds justify the necessity to study the variability of effect of fungicides on the legume-rhizobia symbiosis. Apron fungicide reduces the viability of *Bradyrhizobium japonicum* (- 61%) on soybean seeds after 1 h incubation (Revellin et al. 1993). Similarly, Captan and pentachloronitrobenzene fungicides reduce the viable *B. japonicum*, - 18% and - 78% respectively, after 1 h exposure (Curley and Burton, 1975). Graham et al. (1980) have observed on Captan-treated seeds that only 10% of *Rhizobium phaseoli* had survived after 24 h contact with fungicide whereas 90% of the strains had survived on the non treated seeds after the same time contact. Nevertheless developing fungicide-resistant rhizobial strains remains one approach to overcome this current constraint for delivering inoculants (Teskai and Mallik, 1986; Hashem et al., 1997).

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## REFERENCES

- Bremner JM (1965). Total nitrogen. In: C. A. Black Methods of soil analysis. Part 2. Agronomy Monograph no. 9. American Society of Agronomy, Madison, pp 1149-1178.
- Brockwell J, Diatloff A, Roughley RJ (1982). Plant infection counts of rhizobia in soils. In: J M Vincent (eds) Nitrogen Fixation in Legumes. Academic Press, New York, pp 41-58.
- Curley RL, Burton JC (1975). Compatibility of *Rhizobium japonicum* with chemical seed protectants. Agron. J. 67: 807-808.
- Diatloff A (1986). Compatibility of systemic and non-systemic fungicides with *Rhizobium japonicum* applied to soybean seed. Soil Biol. Biochem. 18: 121-122.
- Diatloff A (1970). The effects of some pesticides on root nodule bacteria and subsequent nodulation. Aust. J. Exp. Agric. Anim. Husband. 10: 562-567.
- Diouf A, Ndoye I, Spencer MM, Nef-Campa C, Gueye M (1999). Need for inoculation of common bean (*Phaseolus vulgaris* L.) in Senegal and assessment of nitrogen fixation using <sup>15</sup>N isotope dilution technique. Symbiosis 27: 251-257.
- Diouf A, de Lajudie P, Neyra M, Kersters K, Gillis M, Martinez-Romero E, Gueye M (2000). Polyphasic characterization of rhizobia that nodulate *Phaseolus vulgaris* in West Africa (Senegal and Gambia). Int. J. Syst. Bacteriol. 50, 159-170.
- Edminsten KL, Wolf DD, Stromberg EL (1988). Compatibility of metalaxyl with *Rhizobium meliloti* on alfalfa seed to control Pythium damping off. Crop Sci. 28: 568-570.
- Fisher DJ (1976). Effects of some fungicides on *Rhizobium trifolii* and its symbiotic relationships with white clover. Pest. Sci. 7: 10-18.
- Fried M, Middelboe V (1977). Measurement of amount of nitrogen fixed by a legume crop. Plant Soil 47: 713-715.
- Graham PH, Ocampo G, Ruiz LO, Dugue A (1980). Survival of *Rhizobium phaseoli* in contact with chemical seed protectants. Agron. J. 72: 625-630.
- Guene NF (2002). Utilisation des inoculums de rhizobium pour la culture du haricot (*Phaseolus vulgaris*) au Sénégal. Thèse de doctorat 3è cycle. Université de Dakar. 96 p.
- Hashem FM, Saleh SA, van Berkum P, Voll M (1997). Survival of *Bradyrhizobium* sp. (*Arachis*) on fungicide-treated peanut seed in relationship to plant growth and yield. World J. Microbiol. Biotechnol. 13:335-340.
- Hofer AW (1958). Selective action of fungicides on *Rhizobium*. Soil Sci. 86: 282-286.
- Olsen SR., Cole LV, Watanabe FS, Dean LA (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circular of U. S. Department of Agriculture no. 939.
- Rennie RJ, Howard RJ, Swanson TA, Flores GHA (1985). The effect of seed-applied pesticides on growth and N<sub>2</sub> fixation in pea, lentil, and faba bean. Can. J. Plant Sci. 65: 23-28.
- Revellin C, Leterme P, Catroux G (1993). Effect of some fungicide seed treatments on the survival of *Bradyrhizobium japonicum* and on the nodulation and yield of soybean (*Glycine max* L. Merr). Biol. Fertil. Soils 16: 211-214.
- Staphorst JL, Strijdom BW (1976). Effects on rhizobia of fungicides applied to legume seed. Phyto-phylactica 8: 47-54.
- Tesfai K, Mallik MAB (1986). Effect of fungicide application on soybean-rhizobia symbiosis and isolation of fungicide-resistant strains of *Rhizobium japonicum*. Bull. Environ. Contam. Toxicol. 36: 816-826.
- Thomas M, Vyas SC (1984). Nodulation and yield of chickpea treated with fungicides at sowing. Int. Chickpea Newsl. 11: 37-38.
- Vincent JM (1970). A manual for the practical study of the root nodule bacteria. IBP Handbook No. 15. Oxford, Blackwell Scientific Publications, Oxford, England. 164 p.
- Welty LE, Prestbye LS, Hall JA, Mathre DE, Ditterline RL (1988). Effect of fungicide seed treatment and rhizobia inoculation on chickpea production. Appl. Agric. Res. 3: 17-20.