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

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A field experiment was conducted at Bel Air station, in Dakar using ^{15}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}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*

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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 ⁻¹)	Nod. DW (mg pl. ⁻¹)	Pod yield (Mg ha ⁻¹)
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 10^7 *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 ¹⁵N-labelled fertilizer solution, (¹⁵NH₄)₂SO₄ containing 5 atom % ¹⁵N excess to supply 20 kg N ha⁻¹. Unlabelled (NH₄)₂SO₄ 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⁻¹ as a triple superphosphate and 120 kg K ha⁻¹ 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 % ¹⁵N excess (%¹⁵Nae) 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):

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

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⁻¹ and 1.2 Mg ha⁻¹ 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⁻¹ 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⁻¹ and 15.4 kg N ha⁻¹ (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⁻¹ vs 86.2 mg plant⁻¹ (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⁻¹ respectively in the shoot, 54% and 48.9 kg N ha⁻¹ 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 % ¹⁵N excess (%¹⁵Nae), 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.

Rhizobium strains	Plant organs	Fungicide	%N	Total N (kg ha⁻¹)	%¹⁵Nae	%Ndfa	Ndfa (kg ha⁻¹)
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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