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Effects of Inoculation with *Bacillus circulans* and *Azospirillum lipoferum* on crop-yield in field grown maize*

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Abstract

Two N_2-fixing rhizobacteria (*Bacillus circulans* RSA19 and *Azospirillum lipoferum* CRT1) were isolated from maize rhizosphere soil and maize roots respectively.

A field trial, was carried out in Southwest France. Treatments consisted of four levels of N fertilizer (0, 80, 160 and 240 kg N/ha) and inoculation with these bacteria.

Significant increases in: grain yield per ha, grain yield per plant, shoot dry matter, nitrogen of grain and total nitrogen exported with harvest were obtained at intermediate levels of N fertilization.

These results show that there is a potential benefit for bacterial inoculation of maize in temperate regions under intensive cropping.

Introduction

Several free-living bacteria called PGPR (plant-growth-promoting rhizobacteria) can exert beneficial effects on plant growth. The use of non symbiotic nitrogen-fixing bacteria has recently been reviewed (Kloepper et al., 1989) and can be of economical importance since they can lead to higher yields and lower consumption of fertilizers or pesticides.

Particular attention has been given to the genus *Azospirillum*. Azospirilla can fix dinitrogen and are found living in close association with the roots of several grasses and cereals (Döbereiner and Pedrosa 1987). *Azospirillum* inoculation on grain grasses can affect root development (Jain and Patriquin, 1984) and can cause increases in dry matter of vegetative parts of maize (O'Hara et al., 1981), wheat (Mertens and Hess,

*Reviewed

1984) and other crops and in grain yield (Okon and Hadar 1987).

Less work has been devoted to the genus *Bacillus*. Some *Bacillus* species are N_2 fixers. Among them *B. polymyxa* seems to play an important role in the rhizosphere of several crops (Chanway et al., 1988; Rennie and Thomas 1987; Lindberg and Granhall 1984). Very little work has been done with *B. circulans*, an other rhizospheric N_2-fixing species.

The purpose of this study is to evaluate the potential of maize inoculation in temperate regions under intensive cropping, using two nitrogen-fixing bacterial strains, an *Azospirillum* and a *Bacillus*. An experimental field trial with four nitrogen fertilizer levels was set up.

Materials and Methods

Strains. Bacillus circulans RSA19 was isolated from maize rhizosphere soil using the spermosphere model (Thomas-Bauzon et al., 1982). It was shown to have the highest dinitrogen-fixing activity among 32 isolates of *B. circulans* using essentially the protocol described by Heulin et al. (1989). *Azospirillum lipoferum* CRT1 was isolated from maize roots as previously described (Fages and Mulard 1988).

Inoculum preparation of Azospirillum lipoferum CRT1. A microencapsulation in calcium alginate matrix and a dehydration in a convection oven were performed on a 24 hours cell suspension with 10^9 CFU/ml, following the protocol described by Fages (1990). The dried inoculum was put in the furrow at the level of 1.5 10^8 CFU/seed.

Inoculum preparation of Bacillus circulans RSA19. A freeze-dried culture of strain RSA19 was added to the seeds before sowing. These seeds had been previously coated with fresh polysaccharide produced by strain RSA19 on solid medium. At sowing, the number of viable bacteria was 3.10^5 CFU/seed. Due to this low level of viable bacteria a second inoculation with this strain was done when the plantlets had three leaves: 10^8 CFU/plant were then added as a dense suspension in 0.8% KC1.

Maize cultivar. The hybrid was SIRENA (Pioneer Hi-Bred Int.)

Location. Saint-Hilaire (south-west France) on a silty soil with a pH of 8.3.

Design. A complete randomized block design with four replications was chosen. Four levels of nitrogen were tested, 0, 80, 160 and 240 kg N/ha. Three bacterial treatments, one uninoculated control, *Azospirillum* inoculation and *Bacillus* inoculation were used. Each of the 48 plots had five rows and was 5.2 m long.

Nitrogen application. N was applied as NH_4NO_3 in solution at the 3–4 leaf stage.

Tested parameters. Before harvest: number of emerged plants per plot (counts done before N application). At harvest (15 plants harvested at random among the three central rows): fresh and dry matter, nitrogen content of cobs, grain, leaves and stems; 1000 kernel weight.

Statistical analysis. A variance analysis was carried out for each parameter. When the Fisher test gave a probability less than 5%, a least significant difference test was performed (Student test).

Results and Discussion

Effect of nitrogen fertilization. A significant increase from one nitrogen level to another on grain yield and its components as well as on total dry matter was observed when nitrogen was a limiting factor. This is often a necessary condition for having a plant growth response to inoculation, as Omar et al. (1989) have demonstrated in rice field inoculation experiments.

Effect of inoculation. Table 1 gives the effect of inoculation on seven variables. A significant increase in the number of emerged plants, total dry matter and grain yield per hectare ($p<0.05$) was observed by *A. lipoferum* CRT1 inoculation. With *B. circulans* RSA19, an increase in nitrogen exported with harvest ($p=0.067$) and in nitrogen content of grain ($p=0.097$) together with a significant decrease in the number of emerged plants was obtained. The effect on the number of emerged plants is in agreement with literature data for *Azospirillum* inoculation on other seedlings (Hadas

Table 1. Effect of inoculation with *Azospirillum lipoferum* CRT1 and *Bacillus cirulans* RSA19 on grain yield, dry matter production and N content[1] of maize crop

	Non inoculated	Inoculated with	
		A. lipoferum	*B. circulans*
Grain yield (g/plant)	180.1[2]a	196.6a	192.2a
(*Standard error*)	(*5.8*)	(*6.4*)	(*6.2*)
N° plant/Ha	65268b	69152c	60848a
(*Standard error*)	(*991*)	(*788*)	(*1360*)
Grain yield (t/ha)	11.77a	13.31b	11.66a
(*Standard error*)	(*0.46*)	(*0.44*)	(*0.40*)
Total[3] dry matter (t/ha)	14.60a	16.15b	15.05a
(*Standard error*)	(*0.63*)	(*0.76*)	(*0.75*)
1000 kernel weight (g)	290a	294a	304a
(*Standard error*)	(*6.0*)	(*5.4*)	(*4.1*)
% N grain	1.42a	1.42a	1.48a
(*Standard error*)	(*0.03*)	(*0.03*)	(*0.02*)
Total[3] N (g/plant)	2.7a	2.84a	3.08b
(*Standard error*)	(*0.12*)	(*0.13*)	(*0.15*)

[1] Figures given here, are overall values irrespective of the nitrogen level.
[2] Numbers are means of 16 measurements. Means with the same letter are not significantly different, $P<1\%$, except for Total N: $P=6.7\%$.
[3] Excluding roots.

and Okon, 1987). However, the decrease found with *B. circulans* is rather unusual. Further germination test (data not shown) indicated that this strain could have a negative effect on the germination stage of maize.

A detailed analysis within each nitrogen level was performed to improve understanding of the effects of inoculation on the other six variables.

Effect on grain yield. An increase (P=0.080) in grain yield per plant was observed (Fig. 1) at intermediate levels of fertilization (80 and 160 kg N/ha). At 160 kg N/ha, inoculation with *Bacillus circulans* was not accompanied by a significant increase in grain yield/ha (Table 2) probably due to a lower number of plants per plot. At 160 kg N/ha the maximum yield of the experiment was reached with *Azospirillum lipoferum* inoculation (14.4 t/ha). Such a yield was obtained with uninoculated plants for a fertilization level of 240 kg N/ha. It thus appeared that *Azospirillum lipoferum* inoculation was equivalent to 80 kg N/ha. This is a clear demonstration that a rather high level of nitrogen fertilization is necessary to produce the optimum effect of inoculation, and confirms previous observations in field experiments with maize (Okon et al., 1988, Kloepper et al., 1989).

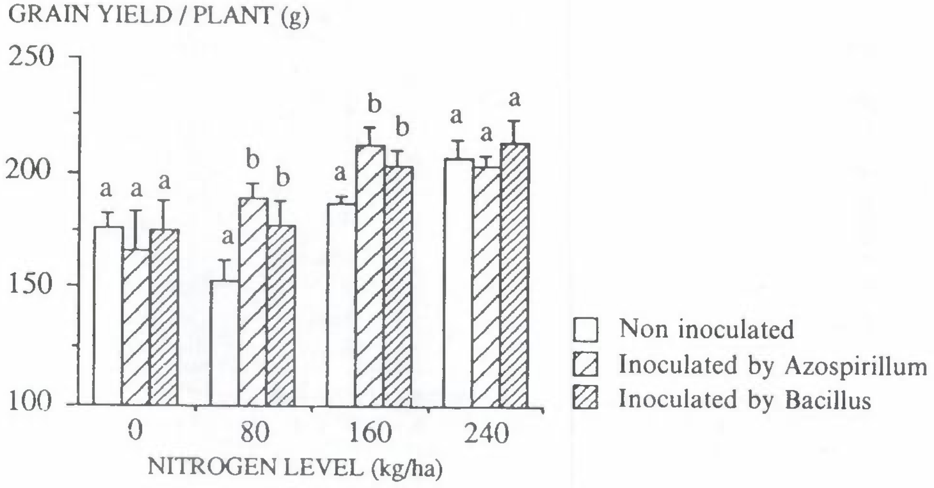

Figure 1. Grain yield per plant of inoculated and non inoculated maize crop at maturity, at four different nitrogen levels.
Bars: standard error of mean. Means with the same letter within each nitrogen level, are not significantly different (0 and 240 kg/ha: P = 5%; 80 and 260 kg/ha: P = 8%).

Effect on nitrogen content of plants. Both inoculations led to increases in nitrogen content of grain (Fig. 2) and in total nitrogen exported with harvest (Table 2) for the

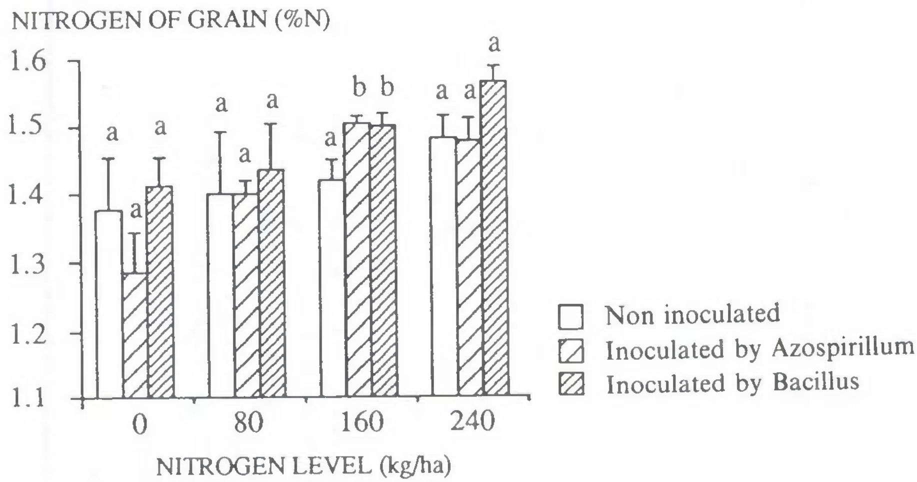

Figure 2. Nitrogen content of grain at harvest of inoculated and non inoculated maize plants, grown at four different nitrogen levels.
Bars: standard error of mean. Means with the same letter within each nitrogen level, are not significantly different (P = 5%).

160 kg N/ha level. Both strains were selected by the spermosphere model (Thomas-Bauzon et al., 1982) for their high ability to fix dinitrogen in association with maize plantlets. This ability could explain such results: the bacteria could have supplied the plants with some extra-nitrogen during the grain filling period. Moreover the production of auxin-like substances by *Azospirillum* strains (Döbereiner and Pedrosa 1987) could have contributed to a better nitrogen nutrition due to a larger soil volume explored by the roots.

Effect on kernel weight. No effect on 1000 kernel weight was observed for either strain (Table 2), although a tendency toward enhancement of this weight could be observed with *B. circulans* inoculation (+4.8%, P = 0.12). However, fewer plants per ha might have led to less competition for nutrients which could explain these results.

Conclusion. These results show that a potential benefit for maize inoculation does exist in temperate regions. The strategic choice of inoculation with strains coming from the plant-host rhizosphere appears to be right. It is the first time that increases in maize grain yield have been obtained at such high levels (14.4 t/ha) with a rhizosphere strain of *A. lipoferum*. The *B. circulans* strain seems to have great potential for improving the nitrogen content of plants at harvest.

Over three years and in several locations, similar results have been obtained with the *A. lipoferum* CRT1 strain (unpublished data):

- grain yield of inoculated plants at 160 kg N/ha equivalent to those of control plants at 240 kg N/ha (1 year, 1 location),

Table 2. Effect of inoculation with *Azospirillium lipoferum* CRT1 and *Bacillus circulans* RSA19 on grain yield, dry matter production and N content of maize crop at four nitrogen levels.

	N level											
	0			80			160			240		
	Non inoculated	Inoculated[1] with A.l.	Inoculated[1] with B.c	Non inoculated	Inoculated with A.l.	Inoculated with B.c.	Non inoculated	Inoculated with A.l.	Inoculated with B.c.	Non inoculated	Inoculated with A.l.	Inoculated with B.c.
Grain yield (t/ha)	10.67a[2]	11.52a	10.65a	10.01a	13.17c	10.87b	12.26a	14.41b	12.11a	14.13a	14.13a	13.00a
(*Standard error*)	(*0.43*)	(*1.29*)	(*0.86*)	(*0.44*)	(*0.28*)	(*0.16*)	(*0.30*)	(*0.35*)	(*0.79*)	(*0.60*)	(*0.56*)	(*0.76*)
Total[3] dry matter (t/ha)	13.60a	14.08a	13.94a	12.47a	15.65c	13.70b	14.71a	17.53a	15.26a	17.63a	17.35a	17.32a
(*Standard error*)	(*0.85*)	(*1.44*)	(*0.95*)	(*0.84*)	(*0.55*)	(*0.40*)	(*0.31*)	(*0.54*)	(*1.38*)	(*1.24*)	(*0.69*)	(*1.08*)
1000 kernel weight (g)	288a	268a	292a	275a	294a	301a	286a	305a	306a	312a	310a	316a
(*Standard error*)	(*3.4*)	(*13.4*)	(*10.7*)	(*16.9*)	(*4.3*)	(*6.2*)	(*12.9*)	(*6.4*)	(*4.1*)	(*2.2*)	(*2.1*)	(*7.9*)
Total[3] N (g/plant)	2.58a	2.22a	2.69a	2.36a	2.70a	2.73a	2.75a	3.28b	3.23b	3.25a	3.16a	3.68a
(*Standard error*)	(*0.19*)	(*0.30*)	(*0.28*)	(*0.24*)	(*0.09*)	(*0.33*)	(*0.09*)	(*0.11*)	(*0.07*)	(*0.24*)	(*0.13*)	(*0.20*)

[1] A.l. = *Azospirillum lipoferum* strain CRT1; B.c. = *Bacillus circulans* strain RSA19.

[2] Numbers are means of 4 replicates. Means with the same letter within each nitrogen level are not significantly different, $P < 5\%$.

[3] Excluding roots.

- earlier flowering for intermediate levels of N fertilization and not for the full dose of 240 kg N/ha (2 years, 2 locations),
- significant increases in number of plants per plot (2 years, 3 locations).

Moreover, these data confirm previous studies on rice, sorghum, millet and wheat showing that inoculation of nitrogen-fixing bacteria has an effect on yields under nitrogen-limiting conditions (Omar et al., 1989, Smith et al., 1984) and that this effect is more pronounced at intermediate levels of N fertilization. There is no antagonistic effect between nitrogen fertilizers and bacterial inoculation, a minimum amount of fertilizer being necessary for yield enhancement through inoculation. There is a hope that PGPR inoculation associated with right rates of N fertilization can produce high yields while saving fertilizers and diminish the risk of water pollution through nitrate leaching.

Regarding mechanisms beside the nitrogen-fixing ability of both strains and the phytohormone production of the *Azospirillum* strain, biological control effect of *Bacillus* cannot be excluded (De Ming and Alexander 1988). The antibiotic production of strains belonging to this genus is well known. *Azospirillum* inoculation seemed to produce effects on early components of grain yield (number of emerged plants), whereas *Bacillus* inoculation seemed to lead rather to late components of yield (1000 kernel weight, N content of grain). A new strategic approach for field inoculation of maize can be proposed from this trial: a seed inoculation with *A. lipoferum* strain followed by an inoculation with *B. circulans* strain performed at a 3–4 leaf stage. This procedure is under current investigation.

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