



Effect of Tilemsi phosphate rock-solubilizing microorganisms on phosphorus uptake and yield of field-grown wheat (*Triticum aestivum* L.) in Mali

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Abstract

With the broad aim of biologically improving P uptake by wheat fertilized with Tilemsi phosphate rock (TPR), we investigated the effect of inoculation with TPR-solubilizing microorganisms isolated from Malian soils and with a commercial isolate of the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* (Gi). AM root length colonization, and growth yield and P concentration of the cultivar Tetra of wheat were measured under field conditions in Mali. Experimental plots were established in Koygour (Diré) during the 2001–2002 cropping season. Inoculation treatments included two fungal isolates, *Aspergillus awamori* (C1) and *Penicillium chrysogenum* (C13), and an isolate of *Pseudomonas* sp. (BR2), used alone or in fungus-bacterium combinations in the presence or absence of the AM fungus Gi. In fertilized treatments, 0 or 30 kg P ha⁻¹ was applied as TPR or diammonium phosphate (DAP). In 45-day-old wheat plants, the highest root length AM colonization (62%) was observed with TPR fertilized wheat inoculated with Gi and BR2. Our results suggest that BR2 is a mycorrhizal-helper bacteria and a good plant growth-promoting rhizobacteria. In fact, inoculation of wheat Tetra fertilized with TPR with a combination of Gi, BR2 and C1 produced the best grain yield with the highest P concentration. This work shows that by inoculating seeds with TPR-solubilizing microorganisms and AM fungi under field conditions in Mali it is possible to obtain wheat grain yields comparable to those produced by using the expensive DAP fertilizer.

Introduction

Phosphorus (P) deficiency is one of the major constraints to crop production in West Africa, and in Mali imported fertilizers are very expensive. However the Tilemsi phosphate rock (TPR) deposits are estimated to be between 20 and 25 million tonnes, and are a potential inexpensive source of P for farmers (Bationo et al., 1997). In fact, economic evaluation of TPR under farmers' operating conditions for three cropping rotations

(groundnut/pearl millet; cotton/sorghum and cotton/maize) clearly indicated that the direct application of TPR could be profitable in comparison with recommended imported P fertilizers (Bationo et al., 1997).

Many bacteria (Rodriguez and Fraga, 1999) and fungi (Whitelaw, 2000) are able to improve plant growth by solubilizing sparingly soluble inorganic and organic phosphates in the soil. Production and release of organic acids is an important mechanism involved in inorganic P solubilization (Richardson, 2001). *Penicillium rugulosum* IR-94MF1 isolated from a soil in Venezuela mobilizes inorganic phosphates by producing

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gluconic and citric acids (Reyes et al., 1999). These organic acids are also involved in PR solubilization (Reyes et al., 2001), and inoculation of maize that had been fertilized with Venezuelan Navay PR with IR-94MF1 significantly increased shoot yield and P-uptake by the plants as compared to the uninoculated control (Reyes et al., 2002).

Most crop plants are colonized by arbuscular mycorrhizal (AM) fungi. Besides improving uptake of poorly mobile nutrients, AM symbioses benefit plant growth by other mechanisms of action such as improving drought tolerance, protecting the plant against pathogens or channeling carbon to the soil, thus improving soil aggregation (Sylvia and Chelleni, 2001). Many recent reports show synergistic interactions between phosphate-solubilizing microorganisms and AM, under different experimental conditions. Using transformed carrot (*Daucus carota* L.) roots, Villegas and Fortin (2001) observed that the combination of the P solubilizing *Pseudomonas aeruginosa* with *Glomus intraradices* enhanced the solubilization of sparingly soluble sources of P above the levels reached with each culture alone. In field trials performed in southern Egypt, the highest significant effect on wheat (*Triticum aestivum* L.) yield and phosphorus content, was observed when seeds were inoculated with a mixture of the AM fungus *Glomus constrictum* with two Egyptian fungal isolates *Aspergillus niger* and *Penicillium citrinum* that solubilize phosphate rock (Omar, 1998).

The aim of the present work was to evaluate TPR as a P source for wheat cultivated under field conditions in Mali, and to explore the possibility of improving its value by seed inoculation with TPR-solubilizing organisms. *Pseudomonas* sp. BR2, *Aspergillus awamori* C1, and *Penicillium chrysogenum* C13 were isolated from Malian soils and selected for their high TPR-solubilizing activity. These organisms were used alone or in combination, with or without a commercial AM isolate of *Glomus intraradices* to inoculate wheat seeds.

Material and methods

Microorganisms

Glomus intraradices, was supplied as a suspension of concentrated pure spores (PremierTech,

Rivière-du-Loup, Quebec, Canada). TPR-solubilizing activity was measured in agar cultures as described by Chabot et al. (1993) by using Goldstein (1986) medium containing TPR as sole source of P. After screening of a large number of rhizosphere isolates obtained from wheat grown in Malian soil, the following organisms were selected for their high solubilization activity and were used in field inoculation trials: *Pseudomonas* sp. BR2, *Aspergillus awamori* Nakazawa C1, and *Penicillium chrysogenum* Thom C13.

Inoculant preparation and seed inoculation

BR2 was cultivated in 50 mL liquid NBRIP medium (Nautiyal, 1999) containing 5 g L⁻¹ TPR as P source, for 48 h on a rotary shaker at 28 °C. Cells were collected and washed three times by centrifugation and suspended in sterile saline. Fungi C1 and C13 were grown on solid TPR-NBRIP medium for 3 and 5 days, respectively. Mycelia and spores from several plates were harvested in 100 mL sterile saline, homogenized in a domestic blender, and washed three times by centrifugation. Seeds of the wheat (*Triticum aestivum* L.) cv. Tetra were surface-sterilized (Chabot et al. 1996), and 200 seeds were soaked in 100 mL of microbial suspension for 4 h at room temperature. The wet seeds were then transferred to sterile plastic bags and mixed by the sequential addition of 20 mL a sterile 1% carboxymethylcellulose solution and 10 g of talc powder. Coated seeds were dried overnight in a laminar flow hood at room temperature. Uninoculated control seeds were treated similarly but without microorganisms.

At sowing each coated wheat seed contained an average of 1.8×10^5 CFU BR2, 1.5×10^2 CFU C1 and 2.1×10^3 CFU C13. The concentrated suspension of spores of *Glomus intraradices* was diluted to 200 spores mL⁻¹ in sterile saline, and 7 mL of this suspension was directly applied in the seed bed in the AM treatments.

Phosphate rock

The TPR deposits contain between 23 and 32% of P₂O₅ and their solubility in neutral ammonium citrate is 4.2% (Bationo et al., 1997). The fine TPR powder used had the following composition (in mg g⁻¹): P, 150; Ca, 329; Al, 20; F, 29.

The extractability of P from TPR determined according to Bolland and Gilkes (1997) was 16.2 mg g⁻¹ in 2% citric acid and 73.4 mg g⁻¹ in 2% formic acid.

Field experiments

Experimental plots were established in Koygour (Diré) during the 2001–2002 cropping season. The 0–15 cm of the silty clay soil at the site had a pH of 6.37 (0.01 M CaCl₂, 1:1 v) and contained 0.17% organic matter. The Mehlich 3 (Mehlich, 1984) available elements (kg ha⁻¹) were as follows: P, 6.3; K, 240; Ca, 804; Mg, 217; Fe, 43 and Al, 255. A split-split plot design was used. The main plots were phosphate fertilization treatments with TPR or diammonium phosphate (DAP) applied at a rate of 30 kg P ha⁻¹ and a non-fertilized control, arranged in randomized complete blocks. The additional N added with the DAP was calculated and compensated for in all other treatments. Sub-plots were inoculated with AM fungus *Glomus intraradices* or uninoculated control. Sub-subplots included the following treatments with TPR-solubilizing microorganisms: *Pseudomonas* sp. BR2, *Aspergillus awamori* C1, and *Penicillium chrysogenum* C13, BR2 + C1, BR2 + C13, and an uninoculated control. The main plots (P fertilization) were 5 m wide and 15 m long. They were divided in two subplots (AM treatments) 2 m wide and 15 m long separated by a 1 m wide buffer zone. Sub-subplots were 2 × 2 m separated by a 60 cm buffer zone, and contained four rows 50 cm apart. Two wheat seeds were planted in each row every 20 cm. Only the two central rows received seeds inoculated with TPR-solubilizing microorganisms. All treatments were replicated four times. Planting was done on November 20, 2001. After emergence plants were thinned to one every 20 cm of row. Nitrogen was applied as 50 kg N ha⁻¹ urea, 2 and 7 weeks after planting which corresponded to stage 2 and stage 5 of Feekes scale (Large, 1954), and a final application of 120 kg N ha⁻¹ urea at stage 10.1 (11 weeks). All plots received 80 kg K ha⁻¹ as KCl. The plots were irrigated 10 times during the growing season (each of approximately 500 m³ ha⁻¹). Plant height was measured 8 weeks (Feekes scale 10) after planting on five randomly chosen plants in the two central rows. Wheat was harvested at maturity on February 18,

2002 (88 days after planting), from a 1-m² area in the center of each sub-subplot.

AM colonization of roots

In the central rows of each sub-subplot, three plants randomly chosen at 45 days after planting, were carefully excavated and their root washed free of soil and stained according to the ink and vinegar technique of Vierheilig et al. (1998), to measure the root length colonization by AM.

Soil and plant analysis

Soil was air-dried and sieved (2 mm) and treated with the Mehlich 3 extractant (Mehlich, 1984) for the determination of available elements. Soil organic matter was estimated by the modified Walkley and Black method (McKeague, 1978). Plant shoots and grain were air dried and weighed, grounded and digested in 15 mL HClO₄ and 5 mL HNO₃. The spectrophotometric vanado-molybdate method was used to measure P (Tandon et al., 1968). Other minerals were determined in plant tissues and soil extracts by atomic absorption spectrophotometry (Gaines and Mitchell, 1979).

Statistical analysis

A three-factor analysis of variance (P fertilization, *Glomus intraradices*, phosphate-solubilizing microorganisms) for each parameter was performed using the general linear models procedure of SAS (1990).

Results and discussion

AM root colonization

P-fertilization and inoculation with phosphate-solubilizing microorganisms (PSM) and *G. intraradices* (Gi) significantly affected root colonization of the cv. Tetra of wheat by indigenous AM fungi (Table 1). All interactions between P-fertilization and inoculation with PSM and Gi were highly significant ($P < 0.001$). This indicates for example, that the colonization by indigenous AM will be affected differently by P-fertilization, according to the applied PSM or the Gi inoculation

Table 1. Summary from the analyses of variance for root arbuscular mycorrhizal colonization (% AM), plant height, grain and shoot yields and P concentrations of wheat cv. Tetra fertilized with Tilemsi phosphate rock or diammonium phosphate (P) and inoculated with different P-solubilizing microorganisms (PSM) in the presence or absence of the AM fungus *Glomus intraradices* (Gi)

| Source of variations | Means squares | | | | | | |
|----------------------|---------------|---------------------|--------------|-------------|---------|-------------|----------|
| | df | % AM | Plant height | Grain yield | Grain P | Shoot Yield | Shoot P |
| Main plots P | 2 | 2132.4*** | 448.1*** | 2.8*** | 2.6*** | 3.9*** | 2.0*** |
| Replications | 3 | 5.7 NS ^a | 95.3** | 0.005 NS | 0.03NS | 0.006 NS | 0.01NS |
| Main plots error | 6 | 2.04 | 25.2 | 0.0003 | 0.03 | 0.0004 | 0.01 |
| Subplots Gi | 1 | 996.2*** | 3145.3*** | 8.5*** | 0.1* | 10.3*** | 1.5*** |
| P × Gi | 2 | 674.4*** | 1.36 NS | 0.05** | 0.3*** | 0.03** | 0.04NS |
| Subplots error | 9 | 1.6 | 27.6 | 0.001 | 0.03 | 0.0003 | 0.01 |
| Sub-subplots PSM | 5 | 2291.1*** | 1143.6*** | 0.9*** | 0.5*** | 1.9*** | 0.2*** |
| P × PSM | 10 | 418.2*** | 100.3*** | 0.1*** | 0.06* | 0.1*** | 0.03* |
| Gi × PSM | 5 | 31.9*** | 150.5*** | 0.4*** | 0.03 NS | 0.3*** | 0.006 NS |
| P × Gi × PSM | 10 | 93.8*** | 96.8 *** | 0.06*** | 0.07* | 0.03*** | 0.007 NS |
| Sub-subplots error | 90 | 3.54 | 18.9 | 0.007 | 0.03 | 0.005 | 0.01 |

*. **. *** Significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

^aNS: Statistically not significant.

treatments. In the uninoculated non-fertilized treatments, indigenous AM fungi colonized only 5.5% of wheat root length, 45 days after planting (Table 2). In semi-arid ecosystems, soil disturbance (grazing, erosion) results in loss of AM propagules and low numbers of viable spores, thus decreasing the mycorrhizal soil infectivity (Diop et al., 1994; McGee, 1989). Inoculation

with Gi in absence of any other treatment did not improve the observed 5.5% AM colonization (Table 2). In pot experiments, Singh and Kapoor (1999) obtained a significant increase in wheat root colonization by inoculation with the AM *Glomus* sp. 88, applied as chopped mycorrhizal root fragments of 10-week-old pearl millet (*Pennisetum typhoides*) and soil. This inoculation

Table 2. Plant height 8 weeks after planting and root colonization by arbuscular mycorrhizal fungi (AM) 45 days after planting of wheat cv. Tetra as influenced by single or dual inoculation with P-solubilizing microorganisms *Pseudomonas* sp. (BR2), *Aspergillus awamori* (C1) and *Penicillium chrysogenum* (C13) in the presence or absence of *Glomus intraradices* (Gi) and by P fertilisation with Tilemsi phosphate rock (TPR) and diammonium phosphate (DAP)

| Inoculation treatments | AM (% colonization) | | | Plant Height (cm) | | |
|------------------------|---------------------|---------|---------|-------------------|----------|---------|
| | Control | TPR | DAP | Control | TPR | DAP |
| Uninoculated | 5.5 c | 8.0 d | 6.5 b | 69.0 c | 73.8 d | 75.0 d |
| BR2 | 43.5 a | 37.8 a | 11.3 a | 83.8 b | 99.5 a | 89.3 bc |
| C1 | 7.3 c | 11.5 bc | 6.0 b | 86.3 ab | 95.0 b | 92.8 b |
| C13 | 9.0 c | 10.3 cd | 6.8 b | 82.0 b | 86.0 c | 84.3 c |
| BR2 + C1 | 26.0 b | 14.0 b | 9.5 a | 91.5 a | 87.8 c | 100.5 a |
| BR2 + C13 | 24.3 b | 13.0 b | 10.8 a | 85.3 b | 86.0 c | 85.8 c |
| Gi | 5.5 d | 25.5 d | 8.8 c | 79.0 d | 85.0 e | 89.3 c |
| Gi + BR2 | 38.5 a | 62.3 a | 14.3 a | 97.3 ab | 106.0 a | 115.0 a |
| Gi + C1 | 11.0 c | 12.5 e | 10.0 c | 90.8 c | 98.0 c | 90.8 bc |
| Gi + C13 | 11.0 c | 12.0 e | 9.0 c | 102.3 a | 93.3 d | 94.0 bc |
| Gi + BR2 + C1 | 24.0 b | 34.5 b | 12.5 ab | 93.0 bc | 100.3 bc | 102.0 b |
| Gi + BR2 + C13 | 22.3 b | 29.8 c | 10.8 bc | 89.0 c | 102.8 ab | 93.8 bc |

For each AM treatment (uninoculated or Gi) within each column means followed by the same letter are not statistically different according to the Fisher protected Lsd test ($P < 0.05$).

procedure probably provided some nutrients not present in the pure spore suspension used in this study. In fact, addition of TPR increased the AM colonization from 5.5 to 8% in the uninoculated control treatment, but a more substantial increase (from 5.5 to 25.5%) was observed in the Gi treated plants (Table 2). In general, inoculation with Gi significantly increased root colonization with AM (Table 3). In general, in the presence of DAP wheat root length colonized with either indigenous or introduced AM fungi was lower than observed in TPR amended or in the unfertilized control plots (Tables 2 and 4). Our results corroborate the observations made by Graham and Abbott (2000) that application of a high rate of soluble P to soil reduces the percentage of root length colonization by AM in 42 day-old wheat plants. The results also agree with the findings of Barea et al. (1980) that phosphate rock does not reduce the level of mycorrhizal infection. Regardless of the phosphorus fertilization treatment, inoculation with the TPR-solubilizing bacterium *Pseudomonas* sp. BR2 significantly enhanced root colonization by indigenous or introduced AM fungi. The highest root length colonization (62%) was obtained with wheat fertilized with TPR and inoculated with Gi and BR2 (Table 2). Inoculation with the TPR-solubilizing *Aspergillus awamori* C1 or *Penicillium chrysogenum* C13 caused less pronounced colonization enhancement of the roots as compared to BR2 (Table 2). The results suggest that BR2 is a mycorrhizal-helper bacterium. Such synergistic interaction between

bacteria and AM fungi is well documented in the literature (Barea et al. 2002).

Plant height

After 8 weeks of growth P-fertilization and inoculation with PSM and Gi significantly influenced the plant height. With the exception of the non-significant P-fertilization \times Gi interaction, all other interactions between P-fertilization and inoculation with PSM and Gi were highly significant (Table 1). For all treatments combined, inoculation with Gi and P-fertilization with TPR or DAP significantly enhanced plant height (Tables 3 and 4). In non-fertilized treatments the highest plant height was recorded when wheat was inoculated with Gi and *Pseudomonas* sp. BR2 or *P. chrysogenum* C13 (Table 2). Plant height also was significantly correlated with grain ($r = 0.70^{**}$, $P < 0.01$) and straw ($r = 0.70^{**}$) yields of mature Tetra wheat.

Grain and shoot yields and P concentrations

Grain and shoot yields and P concentrations were significantly affected by P-fertilization, inoculation with Gi and PSM (Table 1). Interactions between the three treatments were significant for grain and shoot yields. For grain P concentration, the Gi \times PSM interaction was not significant and all interactions involving Gi were not significant for shoot P concentration (Table 1). For all

Table 3. Effect of inoculation with *Glomus intraradices* (Gi) on wheat cv. Tetra height 8 weeks after planting, root arbuscular mycorrhizal colonization (AM), grain and shoot yields and P concentrations

| | -Gi | +Gi |
|---------------------------|--------|--------|
| AM % colonization | 14.5 b | 19.7 a |
| Plant height (cm) | 86.3 b | 95.6 a |
| Grain yield (t/ha) | 2.18 b | 2.67 a |
| Grain P (mg/g dry matter) | 2.30 b | 2.36 a |
| Shoot yield (t/ha) | 2.45 b | 2.99 a |
| Shoot P (mg/g dry matter) | 1.16 b | 1.36 a |

Values are means of P fertilisation and inoculation with P-solubilizing microorganisms treatments. In each line, means followed by the same letter are not statistically different according to the Fisher protected Lsd test ($P < 0.05$).

Table 4. Effect of fertilisation with 30 kg P⁻¹ applied as Tilemsi phosphate rock (TPR) or diammonium phosphate (DAP) on wheat cv. Tetra height 8 weeks after planting, root arbuscular mycorrhizal colonization (AM), grain and shoot yields and P concentrations

| | Control | TPR | DAP |
|---------------------------|---------|--------|--------|
| AM % colonization | 19.0 b | 22.6 a | 9.7 c |
| Plant height (cm) | 87.4 b | 92.8 a | 92.7 a |
| Grain yield (t/ha) | 2.14 b | 2.55 a | 2.57 a |
| Grain P (mg/g dry matter) | 2.08 c | 2.36 b | 2.55 a |
| Shoot yield (t/ha) | 2.44 b | 2.71 b | 3.00 a |
| Shoot P (mg/g dry matter) | 1.08 b | 1.21 b | 1.48 a |

Values are means of all inoculation treatments (P-solubilizing microorganisms and *Glomus intraradices*). In each line means followed by the same letter are not statistically different according to the Fisher protected Lsd test ($P < 0.05$).

treatments combined, inoculation with Gi increased significantly plant and shoot yields and their P concentrations (Table 3). P-solubilizing microorganisms may also directly increase P uptake by changing root morphology. Root hairs can substantially increase root-soil contact, and play a determinant role in P acquisition. Gahoonia et al. (1997) found that the number, length and surface area of root hairs are very variable in wheat cultivars. Gulden and Vessey (2000) also reported that inoculation of field pea (*Pisum sativum* L.) with *Penicillium bilaii* resulted in a 22% increase in the proportion of root containing root hairs and a 33% increase in the mean root-hair length in seedlings. Future work should investigate the effects of inoculation with PSM and AM fungi on root hair development in different cultivars of wheat. Fertilization with DAP increased the four parameters studied as compared to the non-fertilized treatments (Table 4). Except for grain yield, as expected DAP was always superior to TPR. Straw yield and P concentration were not different in the non-fertilized control and the TPR amended plots (Table 4).

In the absence of any P fertilization treatment, inoculation of wheat with the AM fungus Gi produced lower grain yield and P concentration as

compared to the uninoculated control (Table 5). In low P soils, inoculation with aggressive and non-aggressive AM fungi reduced the growth of wheat (Graham and Abbott, 2000). In our study this non-beneficial effect was eliminated by fertilization with TPR or DAP or by inoculation with the P-solubilizing microorganisms tested. On average, inoculation with the AM fungus Gi caused significant increases in grain (0.49 t ha^{-1}) and shoot (0.54 t ha^{-1}) yields for all P-fertilization and PSM inoculation treatments (Table 3). When inoculated with TPR-solubilizing microorganisms, grain yields obtained with TPR treatment were comparable to those produced with DAP (Table 5). When all Gi and PSM treatments are considered the addition of 30 kg P ha^{-1} as TPR or DAP produced 0.42 t ha^{-1} more grain than the unfertilized control (Table 4). Wheat grain yield was always improved by inoculation with PSM in the non-fertilized control and TPR treatments. No grain yield response to inoculation with PSM was observed when DAP was added in the absence of Gi (Table 5). In general grain and shoot yields of wheat inoculated with *A. awamori* C1 or *P. chrysogenum* C13 were always higher when plants were inoculated with Gi as compared to the uninoculated control

Table 5. Wheat cv. Tetra grain and shoot dry matter yields and P concentrations as influenced by single or dual inoculation with P-solubilizing microorganisms *Pseudomonas* sp. (BR2), *Aspergillus awamori* (C1) and *Penicillium chrysogenum* (C13) in the presence or absence of *Glomus intraradices* (Gi) and by P fertilisation with Tilemsi phosphate rock (TPR) and diammonium phosphate (DAP)

| Inoculation treatments | Grain Yield (t/ha) | | | Grain P (mg/g dry matter) | | | Shoot yield (t/ha) | | | Shoot P (mg/g dry matter) | | |
|------------------------|--------------------|---------|--------|---------------------------|--------|---------|--------------------|--------|--------|---------------------------|--------|--------|
| | Control | TPR | DAP | Control | TPR | DAP | Control | TPR | DAP | Control | TPR | DAP |
| Uninoculated | 1.94 d | 2.14 c | 2.23 a | 1.96 d | 1.99 e | 2.09 c | 2.10 e | 2.08 f | 2.25 b | 0.89 e | 1.09 b | 1.19 d |
| BR2 | 2.04 b | 2.35 ab | 2.39 a | 2.01 c | 2.37 b | 2.32 ab | 2.28 b | 2.60 b | 2.94 a | 1.02 bc | 1.12 b | 1.54 b |
| C1 | 2.00 bc | 2.28 b | 2.17 a | 1.99 cd | 2.22 d | 2.28 ab | 2.33 a | 2.37 e | 2.76 a | 0.97 d | 1.03 c | 1.38 c |
| C13 | 1.55 e | 2.32 ab | 2.17 a | 1.97 d | 2.19 d | 2.23 b | 1.89 f | 2.50 d | 2.72 a | 0.99 cd | 1.02 c | 1.18 d |
| BR2 + C1 | 2.12 a | 2.38 a | 2.40 a | 2.17 a | 2.48 a | 2.35 a | 2.15 d | 2.55 c | 2.79 a | 1.08 a | 1.16 a | 1.61 a |
| BR2 + C13 | 1.98 cd | 2.39 a | 2.36 a | 2.11 b | 2.31 c | 2.30 ab | 2.23 c | 2.72 a | 2.87 a | 1.05 ab | 1.09 b | 1.51 b |
| Gi | 1.51 e | 2.14 e | 2.58 c | 1.86 d | 1.20 e | 2.35 b | 2.07 d | 2.10 d | 2.46 d | 1.09 e | 1.30 b | 1.43 a |
| Gi + BR2 | 2.62 b | 2.94 bc | 2.90 a | 2.18 b | 2.57 b | 2.53 a | 2.93 a | 3.22 a | 3.57 a | 1.12 d | 1.37 b | 1.65 a |
| Gi + C1 | 2.60 bc | 2.86 d | 2.98 a | 2.20 b | 2.51 c | 2.52 a | 2.95 a | 2.99 c | 3.59 a | 1.15 cd | 1.29 b | 1.45 a |
| Gi + C13 | 2.12 d | 2.90 cd | 2.72 b | 2.14 c | 2.48 c | 2.49 a | 2.53 c | 3.12 b | 3.17 c | 1.16 c | 1.28 b | 1.48 a |
| Gi + BR2 + C1 | 2.7 a | 3.04 a | 2.95 a | 2.28 a | 2.69 a | 2.51 a | 2.82 b | 3.20 a | 3.46 b | 1.28 a | 1.47 a | 1.71 a |
| Gi + BR2 + C13 | 2.56 c | 2.96 b | 2.93 a | 2.15 c | 2.37 d | 2.51 a | 2.96 a | 3.09 b | 3.49 b | 1.22 b | 1.37 b | 1.68 a |

For each AM treatment (uninoculated or Gi) within each column means followed by the same letter are not statistically different according to the Fisher protected Lsd test ($P < 0.05$).

(Table 5). The positive interaction observed between Gi and PSM like C1 and C13 for shoot yield (Table 1) is comparable to that found with wheat cultivated under field conditions in Egypt, fertilized with phosphate rock and inoculated with *Glomus constrictum* and the two P-solubilizing fungi *A. niger* and *Penicillium citrinum* (Omar, 1998).

Without P-fertilization, the highest grain yields were observed with the BR2 + C1 treatment in the presence or absence of Gi (Table 5). This combination of PSM was also the best for grain yield in TPR fertilized treatments. In addition to its potential as mycorrhizal helper bacteria, *Pseudomonas* sp. BR2 like other PSM used in this study is probably a good PGPR. As the highest grain P concentrations were also observed with the Gi + BR2 + C1 combination in the non-fertilized and the TPR treatments, P-solubilization is probably an important mechanism involved in the observed growth promotion. In fact, a significant increase in P solubilization was observed when an isolate of P-solubilizing *Pseudomonas aeruginosa* was added with the AM fungus Gi to transformed carrot roots (Villegas and Fortin 2001). Similar *in vitro* studies between AM and the phosphate solubilizing fungi deserve to be investigated. *Pseudomonas* strains can also stimulate mycelial development from *Glomus mosseae* spores germinating in soil and tomato root colonization (Barea et al., 1998). Further work should be conducted to evaluate the performance of the Gi + BR2 + C1 combination in different soils and with different wheat cultivars. In fact some PGPR inoculants can adversely affect mutualistic associations between wheat and AM fungi under certain field conditions (Germida and Walley, 1996). The species and type of indigenous AM fungi involved (Graham and Abbott, 2000), wheat genotype (Zhu et al., 2001), and seed P contents (Zhu and Smith, 2001) are additional factors that can also significantly influence this symbiosis.

Concluding comments

This work shows that under field conditions in Mali it is possible to obtain wheat grain yields comparable to those produced by using the expensive DAP fertilizer, by using the less expensive locally available fertilizer TPR, combined

with TPR-solubilizing microorganisms and AM fungi. To make TPR economically profitable to farmers, future work should be oriented towards the development and production of inexpensive formulations of Gi and PSM inoculants, by using locally available material. More field assays in different agricultural regions in Mali are necessary to test the efficacy of the inoculants in the presence of different indigenous soils microbial communities.

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