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Ability of symbiotic and non-symbiotic rhizospheric microflora of maize (*Zea mays*) to weather micas and to promote plant growth and plant nutrition

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Key words Axenic culture Biotite Endomycorrhiza *Glomus mosseae* K uptake Microflora Plant growth Symbiosis Weathering *Zea mays* L.

Summary Maize was grown under axenic conditions in laboratory devices, in a K^+-deficient medium, where biotite was the K^+ source. In different treatments plants were inoculated by symbiotic (*Glomus mosseae*) and/or non symbiotic microflora. In those treatments inoculated by *Glomus mosseae*, the percentage of roots infection after 7 weeks plant growth was 65%. Rhizospheric bacterial population was approximately 10^8/g (dry weight). Endomycorrhizae stimulated growth and K uptake. Non-symbiotic microflora increased also plant growth but promoted much more biotite weathering and K uptake. Endomycorrhizae and more particularly non-symbiotic microflora increased also Ca and Mg absorption by plants. Possible mechanisms involved and implications in plant growth and pedogenesis are discussed.

Introduction

The abilities of root systems to weather minerals were reported by several authors[7,8,15,16,19,28]. These, however, did not distinguish the role of the rhizospheric micro-organisms from that of the plants themselves.

Since the experiments of Gerretsen[10], who observed a better growth of plants inoculated with phosphate-solubilizing bacteria and growing in sand plus rock phosphate, we have few data concerning microbial weathering in the rhizosphere.

Most of the studies concern essentially the effect of micro-organisms on the uptake of soluble mineral elements by plants[3]. Other studies concern counting of microbial population solubilizing phosphates[9,17,25,27,29] or silicates[13]. Generally, the organisms solubilizing insoluble phosphate and silicate were consistently present in higher proportions in rhizosphere isolates than in those from nearby soil. The micro-organisms involved were aerobic or anaerobic.

Some experiments[1,12] gave different types of results and did not show similar effect of inoculation of bacteria in the rhizosphere and other authors[2,23] have shown that vesicular-arbuscular mycorrhizal fungi (*Glomus*) stimulate the uptake of soluble and insoluble phosphate. A bacteria able to solubilize rock phosphate *in vitro*, associated to mycorrhizal fungi, enhanced mycorrhization and phosphate uptake[2]. Inoculation of calcium phosphate dissolving bacteria on seedling cultures of *Pinus resinosa* in a soil deficient in soluble phosphate, but enriched with insoluble calcium phosphate, enhanced seedling growth as well or better than soluble phosphate fertilizer[26].

More recently, it was reported[18] that the inoculation of soybean plants by Glomus endomycorrhizae increases the uptake of K from biotite. But they did not determine the possible role of the non symbiotic microflora that can act on the mycorrhizal root infection[6] and seem also able to play a major role in rock-weathering processes[5] and plant nutrition[3].

This paper reports an experiment performed in order to distinguish the role of non symbiotic, symbiotic and mixed microflora of the rhizosphere of maize on the weathering of a mica (biotite) and on the plant growth.

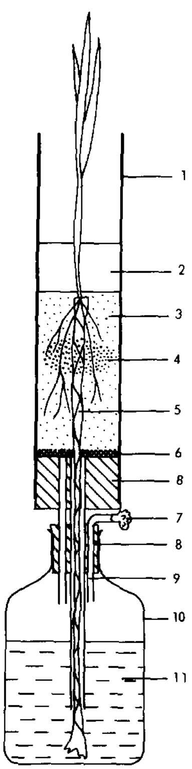

Fig. 1. Experimental device for plant growth.

1. Pyrex tube (∅ 5 cm, high 40 cm)
2. Sand coated with silicone
3. Sand coated with agar
4. Mineral (biotite)
5. Glass fibre
6. Glass wool
7. Cotton plug
8. Rubber stopper
9. Pyrex tube (∅ 8–9 mm)
10. Glass bottle
11. Nutrient solution

Materials and methods

Experimental device

In order to distinguish the role of the plant alone from those of the plant inoculated by an endomycorrhizal fungi (*Glomus mosseae*) or by a complex non symbiotic microflora or by both microflora, an experimental device in which maize grew in axenic conditions was used. We have modified the device that was utilized to determine the extraction of soil potassium by plants[24]. Each device included two main compartments, one supporting the plant, the other containing the nutrient solution (Fig. 1).

Plant compartment Each plant was cultivated in a pyrex tube (400 mm high, 55 mm diameter) that received 375 g of sand coated with agar and 1 g of ground biotite (size 50 to 250 μm) as potassium source. Agar coating was prepared by adding 60 ml of a 8‰ agar noble (Difco) solution to the sand. The chemical analysis of the biotite was as follow (‰): SiO_2, 389; Al_2O_3, 148; Fe_2O_3, 189; MnO, 2.8; MgO, 140; CaO, 7.9; Na_2O, 1.5; K_2O, 87; ignition loss, 32. Previously, the sand was sieved between 0.5 to 1.0 mm, washed by 6 *N* HCl and rinsed 20 times with distilled water.

Each device received a sterile seedling 3 to 5 days old. Sterility of the roots systems was maintained by addition of 100 g of fine sand (100–500 μm) (sable de Fontainebleau Prolabo) coated with silicones and that formed a layer of approximately 40 mm at the top of the sand coated with agar. This 'hydrophobic' sand allowed the gas circulation but retained solid and liquid particles. It was prepared by mixing 100 g of sand to 0.6 ml of hydrofugeant 86 rhodorsil (Rhone-Poulenc) dissolved in 19.4 ml of benzene. The sand was sterilized by autoclaving (30 mn at 110°C) after evaporation of benzene.

Nutrient solution compartment The potassium deficient nutrient solution, described in Table 1, was contained in a one liter bottle connected to the plant compartment by 2 pyrex tubes fixed in rubber stoppers. One of this tube contained glass fibres* that allowed the transfer of nutrient solution to the plant. The second allowed the drainage of the plant compartment and the air circulation. A third tube allowed, through a cotton plug, air circulation with the outside of the bottle.

Table 1. Potassium deficient medium for maize growth (Börner-Rodemachez, modified nutrient medium)

$Ca(NO_3)_2$	0.5 g
NH_4NO_3	0.2 g
NaH_2PO_4	0.2 g
$MgSO_4 \, 7H_2O$	0.25 g
$MnCl_2 \, 4H_2O$	0.005 g
H_3BO_4	0.002 g
Oligoelements solution 1 ml (*)	
EDTA Fe solution 1 ml (**)	
Distilled water 1000 ml	
pH adjusted to 6.4 with 0.1 *N* NaOH	

* Oligoelements solution[14]: boric acid 2857 mg, manganese sulfate 2238 mg, copper sulfate 218 mg, ammonium molybdate 258 mg, zinc chloride 100 ml of a 500 ppm zinc solution, adjusted to 1000 ml with distilled water.

** EDTA solution[11]: disodium EDTA 33.31 g; $FeSO_4 \, 7H_2O$ 21.88 g adjusted to 1000 ml with distilled water, pH adjusted to 5.5 with NaOH (stirring during one night).

* Laine de verre longues fibres, Arts Chimiques, Nancy.

The device compartments, the nutrient solution, the biotite, the sand coated with agar were sterilized separately by autoclaving (2 times 30 min at 120°C). All these parts were put together in sterile conditions (sterile room).

Seed surface sterilization and germination

About 100 maize seeds (*Zea mays* LG 11) were placed in 200 ml H_2O_2 30% and stirred 30 mn. After draining, the seeds were rinsed one time in 200 ml sterile water. To obtain vertical plants and a good sterility control, each seed was germinated, at 28°C in the dark, in a 22 mm diameter tube containing 10 ml of nutrient broth (Difco) with 4‰ agar (Mérieux). Generally, the sterile seedlings were used after 3 or 5 days. This method is successful and respectively the rate of sterility and germination were 90 to 100% and 80 to 90%.

Roots inoculation

The non symbiotic complex microflora was obtained from a 10^{-3} suspension of a maize rhizospheric soil. One ml of the suspension (filtered at 5 to 10 µm to eliminate the fine endophytes) was added to one seedling before addition of sand coated with silicones. In the 'plant alone' treatments and in 'endomycorrhizal plant' treatments, one milliliter of the suspension was also added but after autoclaving (2 times 30 mn at 120°C).

The sporocarps of Glomus were obtained from inoculated onions. The spores dispersed in water were observed with a stereoscopic microscope and collected with microforceps and micropipets. Samples of 50 spores were placed in test tubes closed by a 10 µm mesh polyamide tissue. Then, the spores were sterilized superficially by stirring 15 min in a solution of chloramine T (2%), streptomycine (0.2%), teepol (1 drop). They were rinsed 6 times in sterile distilled water. Fifty spores were used to inoculate one plant.

Plant growth conditions

Plants were grown in one cubic meter sterilized cabinet where temperature, humidity, lighting were controlled during all the experiment. Illumination was of 14 hours and night of 10 hours a day. The light intensity was 20,000 lux (five 400 watts Sylvania BU lamps). Temperature was 28°C and 24°C respectively during illumination and 'night' and humidity 65 to 80% and 85 to 90% during the same periods.

Analysis and controls

Rhizospheric microflora At the end of the experiment, counts of total non symbiotic microflora of the rhizosphere were performed using the usual plate technique with the following medium: peptone 0.5 g; yeast extract 0.2 g; mannitol 1.0 g; K_2HPO_4 0.5 g; glucose 10.0 g; sucrose 4.0 g; soil extract 100 ml; agar 15.0 g, distilled water 1000 ml. Glucose was sterilized separately. The treatments 'plant alone' and 'plant + endomycorrhiza' contaminated with non-symbiotic micro-organisms were eliminated.

The endomycorrhizal infection was verified by microscopic examination of 30 root fragments 1 cm long. After fixation with a solution of formaldehyde (13 ml), acetic acid (5 ml), ethanol 50% (200 ml), staining was done according to the method of[21]. The rate of infection was calculated as the percentage of infected root fragments.

Biomass and chemical plant analysis After a 7 weeks cultivation period, plant growth in the different treatments was compared after the measurement of the shoots dry weight production (shoots were dried during 3 days at 60°C). Then, after grinding, they were mineralized by hydrogen peroxide and perchloric acid. After mineralization, K, Mg, Ca were estimated by atomic absorption spectroscopy (Techtron Varian AA4).

Results

After a 7 weeks growth period, respectively 40 and 70% of the root systems in the treatments 'plant alone' and 'plant + endomycorrhiza' were contaminated by non-symbiotic micro-organisms and were eliminated. Analysis were performed only on the non-contaminated replicates of these treatments.

The endomycorrhizal root infection rates of maize were respectively 67 and 65% in the plant inoculated by *Glomus mosseae* and in the plant inoculated by *Glomus mosseae* and the non-symbiotic microflora. The rhizospheric bacterial population were similar for the plant inoculated only by the non-symbiotic microflora and for the plant inoculated by both microflora (Table 2). Respectively 10^8 and 3.10^7 bacteria per gram of dry rhizospheric material were counted in the considered treatments.

Table 2. Root infection rate of maize by *Glomus mosseae* and bacterial rhizospheric population

	Plant alone	Plant + non symbiotic microflora	Plant + endomycorrhiza	Plant + endomycorrhiza + non symbiotic microflora
Infection rate (%) by *Glomus mosseae*	0	0	67	65
Bacterial rhizospheric population (per g of dry rhizospheric soil)	0	10^8	0	3.10^7

This rhizospheric bacterial population was approximately 10 times more important than the non-rhizospheric bacterial population counted on the non-rhizospheric sand coated with agar.

Shoots growth was stimulated by non-symbiotic and symbiotic microflora (Table 3). Significant differences were observed (Table 4) only between plant alone and plant inoculated by non-symbiotic microflora and between plant alone and endomycorrhizal plant. Inoculation by both microflora did not significantly increase plant growth comparatively to the effect of each separated microflora.

Uptake of potassium, supplied essentially as biotite, was also stimulated by the symbiotic and the non-symbiotic microflora. But more significant increase of the K uptake in the shoots was observed in presence of non-symbiotic microflora that has also promoted a better growth. As for growth and depending of the variability of the results, mixed inoculation by both microflora did not promote potassium uptake significantly.

For mineral elements, supplied in nutrient solution as soluble mineral salts, the presence of rhizospheric micro-organisms promoted uptake of calcium and

Table 3. Influence of rhizospheric microbial populations on maize growth and mineral elements uptake by shoots in presence of biotite as K source (after 7 weeks growth) (mg/plant)

	Plant alone	Plant + non symbiotic microflora	Plant + endomycorrhiza	Plant + endomycorrhiza + non symbiotic microflora
Shoots weight	456 ± 180	979 ± 46	1050 ± 60	1170 ± 334
K uptake	1.5 ± 0.1	2.6 ± 0.1	1.9 ± 0.1	2.8 ± 0.5
Ca uptake	1.8 ± 0.3	4.8 ± 0.6	3.2 ± 0.3	3.7 ± 0.8
Mg uptake	2.1 ± 0.8	4.9 ± 0.3	3.4 ± 0.2	4.8 ± 1.2

K is brought as biotite, but 0.26 mg and 0.7 mg were respectively brought by the seeds and the nutrient solution.
Seed weight: 300 mg

Table 4. Significant differences between treatments

	Plant + microflora	Plant + endomycorrhiza	Plant + microflora + endomycorrhiza
Shoots weight			
Plant alone	*	*	N.S.
K uptake			
Plant alone	*	**	N.S.
Plant + endomycorrhiza	*		N.S.
Ca uptake			
Plant alone	**	*	N.S.
Mg uptake			
Plant alone	*	N.S.	N.S.
Plant + endomycorrhiza	*		N.S.

* $P < 0.01$
** $0.01 < P < 0.05$
N.S. = No significant

magnesium, but significant increases were observed only with plants inoculated by the non-symbiotic microflora for Ca and Mg and with plants inoculated by *Glomus mosseae* for Ca uptake.

But considering the mineral elements concentration in the plants, mycorrhizal plants had lower shoots K, Ca and Mg concentrations than non mycorrhizal plants (Table 5). For plants inoculated only with the non-symbiotic microflora, larger concentrations were observed only for Ca and Mg present as soluble elements in the nutrient solution.

Table 5. Potassium, calcium, magnesium shoot concentration (‰)

	Plant alone	Plant + non symbiotic microflora	Plant + endomycorrhiza	Plant + endomycorrhiza + non symbiotic microflora
K	3.3	2.7	1.8	2.4
Ca	3.9	4.9	3.0	3.2
Mg	4.6	5.0	3.2	4.1

Discussion and conclusion

The culture device for plant, in this experiment, was successfully used to obtain axenic root systems at a relatively good rate. It allowed also a high level of endomycorrhizal infection when maize was inoculated by *Glomus mosseae* and a large development of non-symbiotic rhizospheric bacteria from a rhizospheric soil suspension.

In addition, in such device, analysis of plant excretions, and of minerals would be easier than for those in which soil was used as support for plant growth. This device, therefore, appears as a possible simplified model of 'soil-root system', even if the utilization of a standard nutrient solution, necessary for plant growth, certainly introduced a difference in plant and microbial responses.

The results obtained in this experiment, showed that the non-symbiotic microflora is, as the endomycorrhizal fungi *Glomus mosseae*, able to play a major role in plant growth which is significantly promoted here by each microflora.

Even if mycorrhizal infection was not modified here by non-symbiotic microflora, no cumulative effect was noted in the presence of the mixed rhizospheric microbial population (symbiotic + non-symbiotic).

The plant alone is able to use insoluble potassium from biotite and endomycorrhiza stimulate this K uptake and increase biotite weathering by loss of potassium. But here, it is the non-symbiotic microflora that seems to be able to promote intensively and significantly the mobilization and the absorption of insoluble potassium from biotite by plant. It also promotes uptake of soluble elements such as calcium and magnesium. But except for Ca and Mg in the plant inoculated with the non-symbiotic microflora, shoot mineral elements concentration was, in all the inoculated plants, lower than in the non inoculated plants.

Different microbial mechanisms seem to be involved. As previously mentioned[22], it may be the increased K uptake by *Glomus mosseae* and by the non-symbiotic micro-organisms, that has stimulated a greater growth. But the endomycorrhizal fungi by production of auxin-like substances[4] and/or by a better soil exploration[20], is able, in this non deficient phosphorus medium, to promote maize growth. The non-symbiotic micro-organisms would have a similar activity. In that case, increase of plant growth certainly will modify the chemical equilibrium of potassium solubility so that the plant can act as a potassium sink ('K in the mineral → soluble K → K in the plant').

Bacteria can also increase significantly the soluble mineral element uptake by an ill-defined process that can act also for potassium.

But it is well known that bacteria can solubilize insoluble mineral elements from rocks such as potassium from biotite by metabolic compounds that render it assimilable by plants.

As such mechanisms are of great importance for our knowledge of the soil formation processes, and of the biogeochemical cycles and also for plant nutrition and plant growth, further studies need to be done to define the mechanisms involved.

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