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IMPROVEMENT OF RESPONSE TO LOW WATER AVAILABILITY IN MAIZE PLANTS INOCULATED WITH SELECTED RHIZOSPHERIC MICROBIAL CONSORTIA UNDER DIFFERENT IRRIGATION REGIMES

AMÉLIORATION DE LA RÉACTION À LA BASSE DISPONIBILITÉ D'EAU DES PLANTE DE MAIS INOCULÉES AVEC DES MICRORGANISMES RHIZOSPHÉRIQUES SÉLECTIONNÉS EN DIFFERENT RÉGIMES D'IRRIGATION

MIGLIORAMENTO DELLA RISPOSTA ALLA SCARSA DISPONIBILITÀ IDRICA IN PIANTE DI MAIS INOCULATE CON CONSORZI DI MICRORGANISMI RIZOSFERICI IN DIVERSI REGIMI IRRIGUI

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Abstract

Low water availability for agriculture is a rising problem in temperate countries. The effect of two different rhizospheric microbial consortia on the tolerance to water deficiency of maize was evaluated under controlled watering regimes. One consortium was a mixture of arbuscular mycorrhizal fungi and rhizospheric bacteria isolated under osmotic stress selective pressure; the other consortium was a commercial product. A higher tolerance of plants to water deficiency was observed when roots were inoculated with microbial consortia. Plant gas exchange parameters were positively affected by inoculation, and a improvements of the leaves mineral nutrients content and of the biomass yield were also recorded. The positive effect should be ascribed to an increased roots development more than to an increased uptake from extraradical mycorrhizal hyphae. The use of microbial inoculants appears to be a suitable practice to improve the crop performances under low water availability.
Keywords: *soil, plant growth promoting rhizobacteria, mycorrhiza, maize, drought*

Résumé

La basse disponibilité d'eau pour l'agriculture est un problème actuel dans les pays tempéré, et des nouvelles techniques pour améliorer l'efficacité de l'emploi de cette ressource sont nécessaires. Deux consortia microbiens sélectionnés de la rhizosphère ont été testé pour améliorer la réponse physiologique de plantes de mais à la basse disponibilité d'eau ; l'un était sélectionné pour la tolérance au stress osmotique, l'autre était un produit commercial. On a observé une tolérance améliorée, une majeure quantité de biomasse produite et une concentration

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supérieure d'éléments nutritifs dans les feuilles pour les plantes inoculées. L'amélioration peut être attribuée à un accru développement radical plutôt que à l'adduction des hyphae extraradicales des mycorrhizes. L'utilisation de microorganismes rhizosphériques sélectionnés peut être considéré convenable pour augmenter les productions agricoles sous manque d'eau pour l'irrigation.
Mots clés: *sol, PGPR, mycorrhiza, mais, sécheresse*

Riassunto

La scarsa disponibilità idrica in agricoltura è un problema emergente nei paesi temperati che impone la ricerca di nuove tecniche per ottimizzare l'uso efficiente di questa risorsa. Nel presente lavoro è stato preso in esame l'uso di inoculi di microrganismi rizosferici selezionati per l'induzione di una risposta fisiologica di piante di mais alla carenza idrica. Sono stati testati due consorzi, di cui uno era formato da funghi micorrizici e batteri della rizosfera isolati da un suolo del Senegal e selezionati per la tolleranza allo stress osmotico, l'altro era un prodotto commerciale. E' stata osservata una più alta tolleranza alla carenza idrica nelle piante inoculate. I parametri di scambio gassoso sono stati positivamente influenzati dall'inoculo, e si e' osservato anche un migliore contenuto di elementi nutritivi nelle foglie ed una maggiore crescita delle piante. L'effetto positivo è ascrivibile ad un incementato sviluppo radicale piuttosto che ad un maggiore assorbimento delle ife micorriziche extraradicali. L'uso di inoculi rizosferici selezionati può essere considerato una pratica auspicabile per migliorare la produzione delle colture in condizioni di limitata disponibilità idrica.
Parole chiave: *suolo, batteri rizosferici promotori della crescita, micorrize, mais, siccità*

Introduction

Due to climate changes, scarce water availability is a rising problem for agriculture also in temperate countries, where new strategies are investigated to improve the optimization of the use of this natural resource. Among other approaches, such as improvement of drought tolerance of plants through genetic tools, as well as by biotechnological and agronomical practices, the inoculation with rhizospheric beneficial microorganisms has been proposed; in fact, several soil microorganisms can induce in drought-stressed plants a positive physiological response (Fleury et al., 2010; Bardi and Malusà, 2012).

Arbuscular mycorrhizal fungi (AMF), that establish symbiotic interactions with plant roots, can increase the amount of water taken up from soil; this could be partially ascribed to the larger volume of soil explored by mycelial hyphae with respect to the volume of soil directly explored by plant roots (Augé, 2001; Smith and Read, 2008). Plant-soil water relations and gas exchanges are affected by AMF symbiosis (Augé, 2000). Several plant growth promoting rhizobacteria (PGPR) have also been recognised as capable of alleviating the consequences of drought stress in plants by different mechanisms, such as production of phytormones that enhance root growth and formation of lateral roots and, consequently, nutrients and water uptake, or reduction of ethylene concentration inside the plant by production of ACC-deaminase (Bardi and Malusà, 2012). AM fungi and PGPR can improve the soil structure and water retention properties by forming and stabilizing soil aggregates through deposition of organic compounds such as glycoproteins or exopolysaccharides (Bardi and Malusà, 2012).

Some studies have been reported in which selected AM fungi or rhizospheric bacteria improved plant adaptation to water deficiency conditions as well as to saline soils (Mayak *et al*., 2004; Tao and Zhiwei, 2005). However, the selection of most effective root-colonising AMF and bacterial strains, specifically selected for the tolerance to water stress, to be applied as consortia would be expected to be more adapted to survive and predominate when introduced into a harsh natural environment (Bardi and Malusá, 2012).

In a previous work the roots inoculation with microbial rhizospheric microorganisms (AMF and PGPR) specifically selected for drought tolerance showed a positive effect in maize plants under water deficiency (Zoppellari *et al.*, 2014). The aim of the present work was to check under different watering regimes the physiological response of maize plants inoculated with selected microbial consortia; the behaviour of plants when water was available only in the deep, far from roots, layers of soil was also investigated, to check if a higher water uptake can be ascribed to the extension of mycorrhizal hyphae in deeper layers of soil.

Materials and Methods

The trial was carried out in a greenhouse with mesocosms formed of plastic bags of 1 mc of volume of thoroughly mixed soil, placed in a trench. Soil texture and composition was: sand 76.5%, lime 20%, clay 3.5%; pH 7.5; salinity 3 Ec; P 12.9 mg $\cdot$ 100 g^{-1}; K 7.71 mg $\cdot$100 g^{-1}; Mg 16.1 mg $\cdot$100 g^{-1}; total N 0.13%; total C 0.35% equivalent to 0.6% organic matter. Six maize plants, cv. NK Famoso Syngenta (FAO class 500-600), were grown in each mesocosm. Before sowing, the soil was fertilized with dry manure, at a dose equivalent to 10 t $\cdot$ ha^{-1}.

Plants were treated with two different inoculations: a drought-tolerant rhizospheric consortium (M1), selected and cultured as previously described (Zoppellari et al., 2014), and a commercial consortium (M2). Control plants were un-inoculated (M0). The inocula were applied at sowing to the soil near the seed (10 g $\cdot$ $plant^{-1}$). Sowing was carried out on June 5^{th} and plants were watered from sowing until bloom. Irrigation pipes were positioned at a depth of about 65 cm and on the soil surface. A layer of a tick tissue was also positioned at about 60 cm depth to reduce the possibility of access of roots to the deeper layer, but allowing fungal hyphae to reach it.

The microcosms were subjected to two irrigation regimes: H2= weekly surface irrigation, H4=weekly irrigations alternated on surface and in dept. All the assays were executed in triplicate. In correspondence of the irrigation episode in July, six weeks after sowing, analysis were carried out on plants under water deficiency (7

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days after irrigation) and in the recovery phase (1 day after irrigation) to determine leaf gas exchange parameters: leaf transpiration rate and CO_2 uptake, recorded by an infra-red gas analyzer ADC-LCPro+ system. The measurements were done on three leaves per plant inserted in the central region of the shoot (nodes 4 to 6 from the shoot base).
The biomass yield of each plant was determined by measuring the dry weight of the shoots and roots separately. Plant tissues were dried in oven at 70°C and the weight of each single plant was measured separately. Plant material for the analysis of mineral composition was dried at 60 ºC for 48 h, and ground to pass a 1 mm-sieve. Total nitrogen was determined using the standard Kjeldahl method after wet mineralization (Ostrowska et al. 1991), K content was determined by ICP spectrometry (Cygański, 1997). Data were analyzed by ANOVA with SPSS software; means were compared by LSD multivariate post hoc at $P \leq 0.05$.

Results

Plants inoculated with both M1 and M2 grew more than un-inoculated M0 under H4 regime, corresponding to deep watering; under H2 regime this effect was observed only for M2 plants (Fig. 1A). Inoculation modified the pattern of plant growth: the root system was bigger in inoculated plants resulting in a lower shoot/root ratio (Fig. 1B).

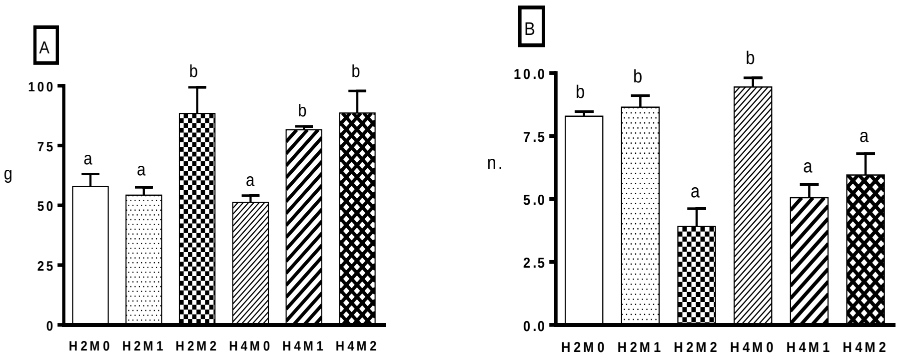

Figure 1 - *Total biomass produced* (A) *and shoot/root ratio* (B) *of maize plants treated with different inocula* (M1, M2) *under two different watering regimes* (H2, H4).
M1: *drought-stress selected microbial consortium;* M2: *commercial microbial consortium;*
H2: *surface weekly watering regime;* H4: *alternated surface/deep weekly watering regime.*

Gas exchange parameters were affected by the inoculation with both consortia. Leaf transpiration rate was higher for M2 in H4 and for both M1 and M2 in H2 than M0 plants (Fig. 2).

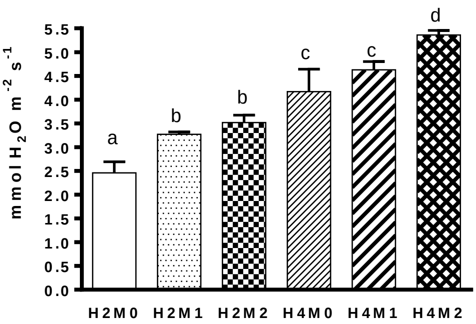

Figure 2
Leaf transpiration rate of maize plants treated with different inoculations (M1, M2) *under two different watering regimes* (H2, H4).
M1: *drought-stress selected microbial con-sortium;*
M2: *commercial microbial consortium;*
H2: *surface weekly watering regime;*
H4: *alternated surface/deep weekly watering regime.*

The commercial inoculum (M2) showed the higher transpiration rate, in particular when water was available in deep layers (H4).
CO_2 uptake was significantly higher in inoculated plants than in un-inoculated ones under low water availability with the surface irrigation regime H2 (Fig. 3A).

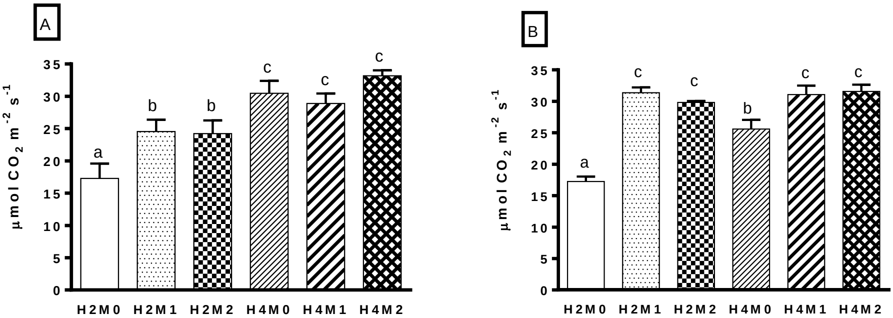

Figure 3 - *CO_2 uptake maize plants treated with different inoculations* (M1, M2) *under two different watering regimes* (H2, H4), *under low water availability* (A) *and during recovery phase after watering* (B).
M1: *drought-stress selected microbial consortium;* M2: *commercial microbial consortium;* H2: *surface weekly watering regime;* H4: *alternated surface/deep weekly watering regime.*

The lowest value was detected for M0 plants under water deficiency (17.3 ± 2.1 mmol µmol CO_2 m^{-2} s^{-1}). During the recovery phase, the inoculated plants showed a significant increase of CO_2 uptake when the H2 irrigation regime was applied (Fig. 3B). These results indicate a higher photosynthetic capacity for the inoculated maize plants in comparison to un-inoculated ones. The inoculation with both consortia improved nutrient uptake (Fig. 4) but differently for the two consortia.

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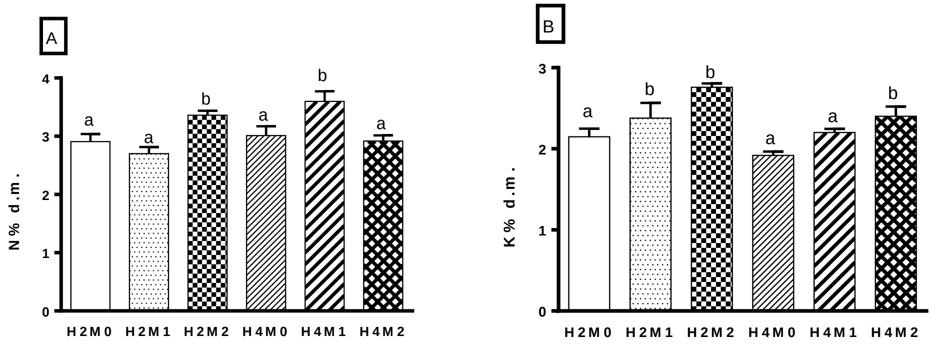

Figure 4 - *Nitrogen* (A) *and potassium* (B) *content of leaves from maize plants treated with different inoculations* (M1, M2) *under two different watering regimes* (H2, H4). M1: *drought-stress selected microbial consortium;* M2: *commercial microbial consortium;* H2: *surface weekly watering regime;* H4: *alternated surface/deep weekly watering regime.*

In fact, nitrogen content was higher with the drought-selected consortium M1 under the H4 deep irrigation regime, while the commercial consortium M2 performed better with the H2 surface irrigation regime (Fig. 4A). The potassium content of leaves was higher in plants inoculated with both consortia in case of H2 irrigation regime, in comparison to the control (Fig. 4B). However, when the H4 deep irrigation regime was applied, the commercial consortium M2 was again better performing in comparison to M1 (Fig. 4B).

Discussion and conclusions

The application of microbial consortia to maize plants affected their physiology, that however were differently performing depending on the irrigation regime applied. The consistent increase of the gas exchange parameters of the inoculated M1 and M2 plants in comparison to M0 is confirming earlier studies showing a significant impact of rhizospheric microorganisms on the physiology of the plant not only at the root level (Augé, 2001). In our trial, when compared to un-inoculated plants, the inoculated plants were differently responding to both high and low water availability conditions. The plants inoculated with the drought-selected microorganisms were showing a significantly better value of the measured leaf gas exchange parameters particularly when water was available in surface. Previous studies have suggested that extra radical hyphae (Allen, 1991) or increased root branching (Kothari *et al.*, 1990) may allow mycorrhizal roots to more fully explore a particular soil volume and increase the access to available water. This was demonstrated delivering water to a hyphal compartment of soil where the penetration of roots was not allowed (Ruiz-Lozano and Azcon, 1995). Moreover, AMF can affect the point of stomatal closure under drought stress (Augé et al., 1992). The use of different AMF species were shown to affect differently the maize plant water relations: inoculation with *G. intraradices* increased leaf gas exchange parameters (Subramanian et al. 1995), while the use of other *Glomus* species did not (Osonubi 1994). These reports and our results are therefore confirming that rhizospheric microorganisms effects on leaf transpiration rate can differ among different microbial species. Some studies have recognised PGPR as capable of alleviating the consequences of drought stress in plants (Yang *et al.,* 2009). Sandhya *et al.* (2010) found that inoculation of maize seeds with *Pseudomonas* spp. under drought conditions improved plant biomass and several parameters related to plant water status. PGPR can also indirectly improve the plant response to drought stress by promoting root development (Yang et al. 2009). This could explain the low shoot/root ratio observed in inoculated plants (Figure 1B). As no significant differences were observed in physiological and growth response of inoculated plants to water availability in surface (H2) or in deep soil layers (H4), the better plants status should not be ascribed to a higher water uptake from extraradical mycorrhizal hyphae, but to a direct effect of rhizospheric microorganisms on an increased root development, as confirmed by the low shoot/root ratio (Figure 1B), as well as on plant physiology and soil physical structure.

The inoculation with both consortia resulted in a higher content of N and K in the plants (Fig. 4). In dry soils, reduced uptake of nutrients might be due to impaired roots functioning or/and reduced nutrient mobility. The effect of inoculated rhizospheric microorganisms on root development and modified plant/water relationships is thus assuring a higher availability and uptake of mineral nutrients. Inoculation of maize plants with *G. intraradices* showed an increase in N leaf content in drought stressed plants (Subramanian and Charest, 1998), but changes of the other elements had not always towards an increasing trend (Subramanian *et al.,* 1997).

The overall effect of the inoculation with the beneficial microrganisms on plant biomass was positive, inducing an increased production of biomass (Fig. 1). A positive effect of AMF on growth and yield of silage maize was recorded under different irrigation regimes particularly when the lowest level of water was provided (Celebi *et al.,* 2010). In our trial, the commercial consortium was effective with both irrigation regimes, while the drought-tolerant one performed better than the control only with the deep irrigation regime. It must be underlined that the inoculation resulted in an increase in the rate of root colonization, irrespective of the consortium utilized (data not shown), but a higher colonization rate was observed for the commercial inoculum in comparison to the drought-tolerant one (Zoppellari *et al.,* 2014). This could account for both the higher photosynthetic capacity and the higher biomass production of M2 plants.

In conclusion, M1 and M2 plants response to water availability appeared better than un-inoculated plants, without significant differences due to the availability in surface or in deep layers. Increased biomass production in inoculated plants derived from water and nutrients uptake and photosynthetic activity also under water deficiency conditions. Such response has also been observed in other inoculated plants (Bethlenfalvay *et al.,* 1987; Ruiz-Lozano and Azcòn, 1995) under water deficiency stress and it agrees with the idea that selected rhizospheric

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microorganisms may be even more important for plants under drought conditions. Then inoculation with rhizospheric microorganisms consortia, either specifically selected for low water availability conditions or not, is a potential tool to be applied in agriculture to improve drought stress tolerance of cultivated plants. Such approach would allow to obtain high crop yields also under not optimal water availability conditions and improve the resource efficiency in agricultural processes.

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