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Mycorrhiza and heavy metal resistant bacteria enhance growth, nutrient uptake and alter metabolic profile of sorghum grown in marginal soil

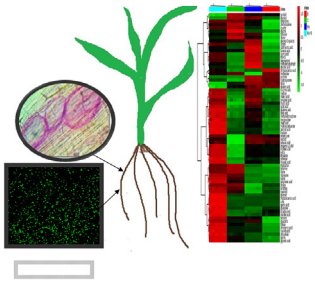
Faten Dhawi [a, b], Rupali Datta [a, **], Wusirika Ramakrishna [a, c, *]

[a] Biological Sciences Department, Michigan Technological University, Houghton, MI, USA
[b] Biotechnology Department, King Faisal University, Saudi Arabia
[c] Centre for Biochemistry and Microbial Sciences, Central University of Punjab, Bathinda, India

HIGHLIGHTS

- Heavy metal resistant bacteria increased sorghum biomass grown in marginal soil.
- Arbuscular mycorrhiza enhanced uptake of most elements by sorghum.
- Dynamic changes in host metabolic pathways regulated by mycorrhiza and PGPB.

GRAPHICAL ABSTRACT



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ABSTRACT

The main challenge for plants growing in nutrient poor, contaminated soil is biomass reduction, nutrient deficiency and presence of heavy metals. Our aim is to overcome these challenges using different microbial combinations in mining-impacted soil and focus on their physiological and biochemical impacts on a model plant system, which has multiple applications. In the current study, sorghum BTx623 seedlings grown in mining-impacted soil in greenhouse were subjected to plant growth promoting bacteria (PGPB or B) alone, PGPB with arbuscular mycorrhizal fungi (My), My alone and control group with no treatment. Root biomass and uptake of most of the elements showed significant increase in all treatment groups in comparison with control. Mycorrhiza group showed the best effect followed by My + B and B groups for uptake of majority of the elements by roots. On the contrary, biomass of both shoot and root was more influenced by B treatment than My + B and My treatments. Metabolomics identified compounds whose levels changed in roots of treatment groups significantly in comparison to control. Upregulation of stearic acid, sorbitol, sebacic acid and ferulic acid correlated positively with biomass and uptake of almost all elements. Two biochemical pathways, fatty acid biosynthesis and galactose metabolism, were regulated in all treatment groups. Three common pathways were upregulated only in My and My + B groups. Our results suggest that PGPB enhanced metabolic activities which

* Corresponding author. Centre for Biochemistry and Microbial Sciences, Central University of Punjab, Bathinda, India.
** Corresponding author.
E-mail addresses: rupdatta@mtu.edu (R. Datta), wusirika@gmail.com (W. Ramakrishna).

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resulted in increase in element uptake and sorghum root biomass whether accompanied with mycorrhiza or used solely.

1. Introduction

The utilization of marginal lands are increasingly being considered as crucial for the production of the second generation of bioenergy crops, which can provide a viable alternative to the use of prime agricultural land (Kang et al., 2013). However, plants grown in these soils have reduced biomass and nutrient deficiency (Brown and Chaney, 2016). Deficiency of nitrogen, phosphorus, and other macro- and micronutrients in soil results in significant loss of plant productivity and affects the quality and quantity of biomass (Shanker et al., 2005). Several studies have reported on the role of microbial interactions in improving soil rhizosphere, availability of macro and micronutrients and increase in plant tolerance (Li et al., 2014; Augé et al., 2014).

Sorghum is a C4 plant with a wide range of adaptations and resistance to several adverse biotic and abiotic factors (Serna-Saldívar et al., 2012). Sorghum is a major staple food crop in many developing countries in the semi-arid tropics of Africa, Asia and Central America and is now a biofuel source in countries such as the U.S., with 30% grain use for ethanol production (Serna-Saldívar et al., 2012). In addition, sorghum growing in heavy metal contaminated soil showed phytoextraction ability (Zhuang et al., 2009). Significant increase in heavy metal tolerance, mycorrhizal colonization, shoot length and total biomass was reported when sorghum was inoculated with plant growth promoting bacteria (PGPB) (Duponnois et al., 2006). Also, the use of PGPB in heavy metal contaminated and nutrient poor soil increased plant growth and metal tolerance of maize (Li and Ramakrishna, 2011).

Earlier studies have demonstrated that the use of a combination of mycorrhiza and PGPB confer several positive effects on plants. These include an increase of mycorrhizal symbiosis, decrease in plant disease symptoms (Jäderlund et al., 2008) and increase in plant biomass (Rajesh Kannan et al., 2011). To the best of our knowledge, the relation between microbial interactions and sorghum biomass, nutrient uptake and metabolic changes has not been investigated. Metabolomics can provide a glimpse of dynamic changes in metabolic pathways in the host plant regulated by mycorrhiza and PGPB. In order to study the influence of microbial interactions on sorghum growth, nutrient uptake and metabolic profile, sorghum roots were subjected to three treatments: mycorrhizal mix, mycorrhiza + PGPB, and PGPB alone. The changes in biomass, element uptake and metabolites were investigated in comparison with untreated group (control).

2. Material and methods

2.1. Plant growth conditions and treatments

The effect of PGPB and mycorrhiza on sorghum biomass and nutrient uptake was studied in marginal soils collected from Lake Linden (Upper Peninsula, Michigan). Two day old germinated seedlings of sorghum BTx623 (USDA) were planted in the greenhouse (30 °C and 65% humidity). Each pot was filled with 600 g of pasteurized soil. Sorghum seedlings subjected to different treatments were classified as the following groups: C (stamp sand with dead inoculum), B (*Pseudomonas* sp. TLC 6-6.5-4), My: mycorrhizal mix, and My + B (*Pseudomonas* sp. TLC 6-6.5-4 + mycorrhizal mix). The PGPB used in this study was isolated from Torch Lake core rich in copper and was found to be resistant to multiple heavy metals and promoted maize growth (Li and Ramakrishna, 2011). The seeds were incubated for an hour for each inoculum before germination and sprayed on the soil surface after germination. Group C represents plants inoculated with 9 g of pasteurized mycorrhiza inoculum powder dissolved in 120 ml of 0.85% NaCl solution. *Pseudomonas* sp. TLC 6-6.5-4 was transformed with rhizosphere stable plasmid pPROBE-GTkan (Miller et al., 2000) according to the transformation protocol described by Krzyzanowska et al. (2012). *Pseudomonas* sp. TLC 6-6.5-4 with *gfp* was grown at 30 °C for 48 h in LB broth supplemented with kanamycin. The bacterial pellet was collected by centrifugation at 6000 rpm for 10 min. Group B represents plants inoculated with 120 ml of *Pseudomonas* sp. TLC 6-6.5-4 (harboring *gfp*) bacterial suspension (10^8 cfu/ml) which was sprayed on the soil surface. Group My represents plants inoculated with 9 g of mycorrhizal mix containing *Glomus intraradices*, *Glomus mosseae*, *Glomus aggregatum*, and *Glomus etunicatum* (100,000 propagules/lb) (Valentine Country Inc., ND, USA), dissolved in 0.85% NaCl. Group My + B represents plants treated with both PGPB and mycorrhiza.

2.2. Plant analyses

Sorghum plants were harvested after 90 days for various analyses. Total chlorophyll content was estimated by atLEAF digital chlorophyll meter. Root and shoot fresh weight was recorded. These samples were dried at 70 °C for 24 h for measuring dry weight biomass. Plant biomass and nutrient uptake was carried out as described below (Motsara and Roy, 2008) and extraction of metabolites was performed according to Lisec et al. (2006). Uptake of the following elements: P, K, Ca, Mg, S, Zn, Mn, Cu, Fe and Al, in roots and shoots was analyzed using inductively coupled plasma mass spectrometry (ICP-MS) to evaluate the effect of different treatments. Biomass and element uptake were evaluated using three replicates for each treatment. Metabolites extraction was performed using a protocol modified from Fiehn (2006) and analyzed by ALEX-CIS GC-TOF MS (West Coast Metabolomics Center, University of California, Davis) with three replicates for each group. Rtx5Sil-MS column (Restek Corporation, USA, 30 m length x 0.25 mm internal diameter with 0.25 μm film made of 95% dimethyl/5% diphenylpolysiloxane) was used with ribitol as an internal standard. (Fiehn et al., 2008). Metabolite data was acquired at 50–330 °C with 1 ml min^{-1} and 0.5 μL injection volume. Helium was used as the mobile phase.

2.3. Localization of mycorrhiza and PGPB

The growth of *Pseudomonas* sp. TLC 6-6.5-4 harboring the rhizosphere stable plasmid pPROBE-GTkan tagged with GFP was evaluated in the presence and absence of kanamycin to estimate the added PGPB only and rhizospheric bacteria plus PGPB, respectively. Mycorrhiza images were taken with a 40 DIC (0.65–100/0.17) trinocular microscope (Nikon, Optiphot, Japan) using digital camera Nikon E 8800 with 10× optical zoom. Successful GFP tagging was identified by visualizing labeled *Pseudomonas* sp. TLC 6-6.5-4 in LB agar with transilluminator (Dark Reader, Clare Chemical Research)