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Growth and Symbiosis of Plants with Arbuscular Mycorrhizal Fungi in Soil Submitted to Biochar Application

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Abstract Arbuscular mycorrhizal fungi (AMF), which is intrinsically present or may be introduced in soils by inoculation, is an example of natural and renewable resource to increase plant nutrient uptake. This kind of fungi produces structures (hyphae, arbuscles and sometimes vesicles) inside the plant root cortex. This mutualistic relationship promotes plant gains in terms of water and nutrient absorption (mainly phosphorus). Biochar can benefit plant interaction with AMF, however, it can contain potentially toxic compounds such as heavy metals and organic compounds (e.g. dioxins, furans and polycyclic aromatic hydrocarbons), depending on the feedstock and pyrolysis conditions, which may damage organisms. For these reasons, the present work will approach the impacts of biochar application on soil attributes, AMF-plant symbiosis and its responses in plant growth and phosphorus uptake. Eucalyptus biochar produced at high temperatures increases sorghum growth; symbiosis with AMF; and enhances spore germination. Enhanced plant growth in the presence of high temperature biochar and AMF is a response of root branching stimulated by an additive effect between biochar characteristics and root colonization. Biochar obtained at low temperature reduces AMF spore germination; however it does not affect plant growth and symbiosis in soil.

Introduction

It is estimated that economically viable phosphate rock reserves, with the current mining technology, will last for up to around 80 years (USGS, 2010). The case of phosphorus (P) is critical, since it is a macronutrient for plants, and most soils are depleted in this element. Moreover, the reserves of P rock are mostly found in only six countries, which could control and limit the use of phosphate for other nations in the future.

Arbuscular mycorrhizal fungi (AMF), which is intrinsically present or may be introduced in soils by inoculation, may help plants to acquire mainly phosphate when a symbiotic relationship between fungi and plant is established. A soil management that benefits AMF may increase the chances of plant colonization by the fungi.

Biochar, which is obtained by pyrolysis of biomass, is a material that is intended to be applied in soils to increase its fertility and enhance plant production. Warnock et al. (2007) point out that biochar application to the soil can benefit plant interaction with AMF, but the extension of its effect is dependent upon biochar characteristics. For this reason, it is important to investigate the effect of different biochars on plant-AMF symbiosis.

The aim of this study is to evaluate soil characteristics, plant growth and the interaction of Dwarf Sorghum plants with AMF in a soil submitted to addition of different biochars.

Materials and methods

The biochars were produced by slow pyrolysis of *Eucalyptus* wood chips (heating rate of 1 °C/min) at 300 °C and 700 °C, and 1 hour of highest heating temperature.

An acidic soil with low organic matter content was collected in Okinawa Prefecture, Japan. The soil was sterilized by autoclaving at 121 °C for 20 minutes. The available P was negligible in soil and biochars (0.49 and 0.01 mg kg^{-1}, respectively) according to Truog (1930). The two biochars were applied at 1% (w/w) rate (plus a control treatment with no biochar) and inoculated by adding 0.5% (w/w) of a commercial AMF inoculant containing *Glomus spp.* (Doctor Kinkon). Non-inoculated pots were used as control treatment. The experiments were carried out in a growth chamber at 25 °C, photoperiod of 14 hours of light, in four replications (3 x 2 factorial experiment with twenty four pots in total).

Since the chosen inoculant contained P in its composition, the inoculant application rate provided a total of 20 mg kg^{-1} P. The sterilized inoculant was added in the treatments without inoculation in order to maintain the same P rate in all pots. Both nitrogen ($Ca(NO_3)_2.4H_2O$) and potassium (K_2SO_4) were applied at a 100 mg kg^{-1} rate. Ten milligrams per kilogram of magnesium ($MgSO_4.7H_2O$) were also added.

Plants were harvested after eight weeks, shoot and root fresh weight were recorded and dry matter was measured after drying the plants for 48 h at 65 °C. Part of the fresh roots were cut into small pieces, stained with trypan blue and submitted to colonization measurement by the gridline intersection method (Brundrett et al. 1996). The root length was assessed after root scanning and image processing by ImageJ freeware. A portion of the soil was analyzed in terms of P sorption capacity, termed P retention (Blackmore, 1987); P availability (Melich-1 extractant) and pH (1:2.5, soil:water). The higher the P retention, the higher the soil capacity to adsorb P.

For the MANOVA the residual normality and homoscedasticity were evaluated and, when necessary, data was transformed (log_{10}). The means were compared by Tukey's test ($p<0.05$). Additionally Person's correlation analysis was applied.

Results and Discussion

The interaction biochar and inoculation factors was not statistically significant. Inoculation did not influence plant growth and soil evaluated parameters (P retention, P availability and pH), however the 700 °C biochar increased all the evaluated plant parameters (Table 1).

Table 1 Overall means for root length (RL) and shoot (fresh and dry) weight of dwarf sorghum plants; phosphorus (P) retention, available P (Melich-1) and pH of a soil inoculated and non-inoculated with arbuscular mycorrhizal fungi under addition of biochars produced at 300 °C and 700 °C

Treatments	SFW†	SDW‡	RL	P retention	Available P [ns]	pH
	g	g	cm	%	mg dm^{-3}	-
300 °C	0.365 b	0.115 b	87.2 b	12.0 b	6.9	4.4 b
700 °C	0.724 a	0.187 a	363.1 a	12.5 ab	4.5	4.8 ab
Control (no biochar)	0.338 b	0.108 b	88.6 b	13.3 a	7.0	4.4 a
Inoculated [ns]	0.480	0.131	197.9	12.6	6.8	4.6
Non-inoculated [ns]	0.471	0.143	161.4	12.6	5.4	4.5

ns: non-significant; means followed by different letters in the columns differ by the Tukey's test ($p \leq 0.05$)
† SFW: shoot fresh weight
‡ SDW: shoot dry weight

Since there were no statistical differences between the biochars for P retention and availability, thus the observed different effect of each biochar in terms of plant growth could be attributed to differences in root elongation and AMF colonization (Figure 1).

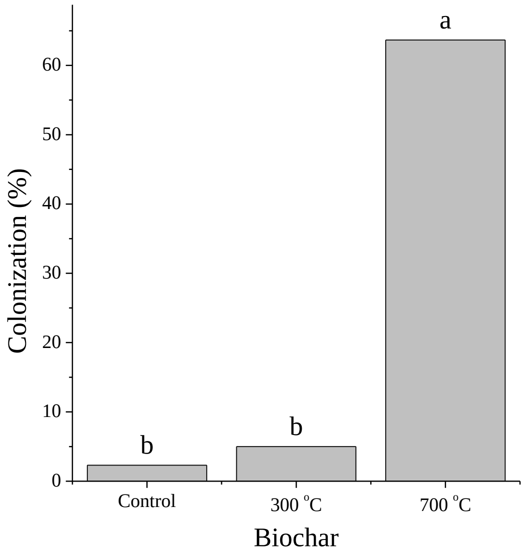

Fig. 1 Colonization of dwarf sorghum plants harvested at eight weeks after plant emergence, submitted to the addition of biochar (control: no biochar). Columns with different letters differ by the Tukey's test ($p \leq 0.05$).

The colonization was highly correlated to soil pH (r=0.96). Both were also positively correlated to plant shoot weight (fresh and dry) and root length, with a minimum "r" of 0.8. This explains why the 700 °C biochar was more effective at promoting root elongation and increasing plant shoot weight. The higher the pyrolysis temperature, the higher ash, pH and nutrient content in biochar which might increase root length. This biochar also enhanced hyphae growing from *Gigaspora margarita* spores. Longer root and hyphae structures raises the probability of interaction between plant and fungi.

Regarding colonization, biochar promotes root branching, fact that combined with AMF hyphae present inside root cortex intensify water and nutrient uptake, alleviating P deficiency symptoms (Smith and Read, 1997) as it was showed by plants during the early growth. Forty days after germination, the plants cultivated under inoculation with AMF and treated with biochar 700 °C started to turn green from purple (purple color means P deficiency). The color changing may coincide with the beginning of symbiosis.

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