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IMPACT OF MYCORRHIZAL FUNGI AND RHIZOSPHERE MICROORGANISMS ON MAIZE GRAIN YIELD AND CHEMICAL COMPOSITION

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ABSTRACT

Yield and grain characteristics of maize plants grown in open-field conditions were evaluated after inoculation with Micosat F® on two different soils under dry and watered conditions. The mycorrhizal frequency and intensity were higher in inoculated maize (87.8 vs 80.3% and 26.8 vs 17.5%, respectively). The abundance of arbuscules in the root system was also higher in inoculated plants (9.7 vs 5.8%). The treatment did not affect grain yield. Positive effect of Typic Eutrudept soil on grain yield was observed. The irrigation effect on grain yield was evident only under draught conditions. Chemical characteristics of grain did not change substantially according to the experimental treatments; nevertheless, the NIRs indicated some physical differences among mycorrhized and not mycorrhized samples. Keywords: Maize grain, root colonization, yield, composition, NIR, electronic nose.

INTRODUCTION

In most of plant species, about 80%, roots can live in symbiosis with arbuscular mycorrhizal (AM) fungi (Smith and Read, 2008), wherein the AM fungi obtain carbon from the plant partner while they transfer mineral nutrients (mostly phosphate) from the soil to the plant root. Advantages derived from AM colonization include enhanced resistance to foliarfeeding insects and soil pathogens, improved resistance to drought (Celebi et al., 2010; Zoppellari et al., 2014), tolerance of salinity and heavy metals and maintenance of soil aggregate stability other than an improved plant nutrition (Gosling et al., 2006). These aspects make the efficient use of AM fungi very appealing to improve plants nutrition. Indeed, it is generally known that the efficiency of phosphorus fertilization is quite low, ranging from 10 to 30% (Gilani, 1983; Isherword, 1998; Gyaneshwar et al., 2002; Hao et al., 2002). Phosphorus deficiency decreases agricultural productivity on more than two billion hectares worldwide (Oberson et al., 2001; Krey et al., 2013). Besides, owing to environmental issues, there is a strong interest to reduce the use of agrochemicals and to save water. Therefore, research must be directed to a sustainable crop yield improvement. The targeted use of AM fungi on cropping may allow the attainment of acceptable yield with minimum fertilizer dose, while reducing production costs and environmental pollution risk (Covacevich et al., 2007). Several studies have stressed the positive effect of AM crop inoculation on growth, nutrient uptake and yield for distinct species such as tomato (Candido et al., 2013), potatoes (Douds, 2007) and many other cereal and vegetable crops under field conditions (Sharm et al., 2007; Hamel and Plenchette, 2007). Plant growth promoting rhizobacteria species (PGPR) are also very effective in promoting plant growth and yield through direct and indirect mechanisms (Günes¸et al., 2014) and they have been proposed as a sustainable component of nutrient management (Richardson and Simpson, 2011; Krey et al., 2013). Bacteria can release growth stimulating hormones, protect against soil-borne pathogens (Vassilev et al., 2006; Krey et al.,2013), improve mineral nutrition (e.g. by increasing plant availability of phosphorus in soil) and they are capable to promote mycorrhiza colonization of maize root as well as maize growth (Krey et al., 2013). Although combined application of PGPR and fungi could be a meaningful approach for sustainable agriculture, there are still certain aspects that need further investigations for obtaining maximum benefits in terms of improved plant growth, particularly under stress conditions (Nadeem et al., 2014). Owing to the multifactorial origin of the interaction between plant and microorganisms, one important aspect is the evaluation of this approach under field conditions. Besides, most field studies evaluated the plant response to single AM fungal strain, while only few reports showed the effects of mixed exotic inoculum (Pellegrino et al., 2011). Responses of plant to exotic inocula are supposed to depend on some factors including: physical and chemical soil characteristics (Davis et al., 1983; George, 2000), native mycorrhizal density, host plant (Pellegrino et al., 2011). Moreover, the effect of inocula on seed characteristics of major crops has been little focused (Berta et al., 2014). More efforts are needed to clarify what strains of fungi, PGPR as well as their combination are most effective in plant helping and benefit and to evaluate what is the effectiveness of co-inoculation in a multi-stressed natural environment for commercialization of microbial inocula (Nadeem et al., 2014). The Micosat F®, patented by an Italian

company, contains symbiotic fungi, rhizosphere bacteria, saprophytic fungi and yeast. The aim of our work was to study the effect of Micosat F® inoculation on maize grown under field conditions. Yield and grain characteristics of mycorrhizal maize plants cultivated in two different soils and under dry and watered conditions were evaluated

MATERIALS AND METHODS

The trial was carried out during two cropping years (2011-12, at the experimental farm of Colle S. Pastore (Rieti, Italy).

Experimental fields: The experimental design included the preparation of eight parcels to study the effects; mycorrhization (present to absent), soil (fine texture to coarse texture) and irrigation (dry to irrigated). Eight parcels delimited by drains, according to the natural allotment, were prepared. The average size of parcels was 0.8 ha. The two soil types utilized were classified according to Soil Taxonomy (Soil Survey Staff, 2003) one Typic Eutrudept with silty loam texture, located in Piedifiume (PF) and the other Vertic Eutrudept with silty clay texture with tendency to surface fissuring (vertic characteristics) on dry season, located in Casabianca (CB) (Raglione et al., 2011). The PF soil characteristics were with pH 8.1, cation exchange capacity 17.9 cmol (+) kg-1 , organic C 9.80 g kg-1 , organic matter 16.80 g kg-1 , N 1.5 g kg-1 , P2O5 30.10 mg kg-1 . The CB soil had pH 7.7, cation exchange capacity 35.7 cmol (+) kg-1 , organic C 14.5 g kg-1 , organic matter 24.9 g kg-1 , N 2.1 g kg1 , P2O5 36.7 mg kg-1 (Raglione et al., 2011). Plots have been subjected to maize monoculture for the last 10 years at least.

Agronomic practices: Ploughing, grubbing and harrowing operations were performed before seeding. Fertilizer was applied at seeding with 92 kg/ha of P2O5 and 36 kg ha-1 of N. An additional dose of 138 kg ha-1 of N as urea was applied at leaf stage. 75000 plants/ha of NK Famoso cv class 500 were sown. Soil geodisinfestation was not performed. MICOSAT F® was applied at sowing (May, 3, 2012) on the four selected fields, according to producer recommendations (15 kg ha-1). MICOSAT F® label composition was three AM species (Glomus caledonium GM24, Glomus intraradices GG31, Glomus coronatum GU53, in form of spores, hyphae and root fragments), and three Plant Growth Promoting Rhizobacteria species (Pseudomonas fluorescens PA28, Pseudo -monas borealis PA29, Bacillus subtilis BA41) with a total concentration of 106 cells g-1 . The inoculum was applied in the same plots during both years. The herbicide Primagram Gold (metolachlor e terbutilazina) was used in preemergence, while in post-emergence, Glitter (nicosulfuron) and Maicol (dicamba) according to the producer recommended doses were administered. Four fields were assigned to dry cropping. For all the others only an emergency sprinkler irrigation was provided at the bloom phase.

Sampling and analyses of roots: Root samples were analyzed to evaluate AM colonization. These were collected only in the second cropping year. The samples were collected in two PF dry parcels (inoculated and uninoculated), at beginning of August. Five strips were obtained from each parcel. Five root systems were sampled from each strip. Three microscope slides per root were prepared. Each slide contained 20 pieces one cm long. In total, 150 slides and 3000 pieces of root were analyzed. Root fragments were stained according to the method described in Novero et al. (2002). Four traits considered were (1) F % (frequency of mycorrhization), reporting the percentage of segments showing internal colonization; (2) M % (intensity of mycorrhization) indicating the average percentage of colonization of root segments; (3) a % (percentage of arbuscules), quantifying the average presence of arbuscules within the infected areas; (4) A % (percentage of arbuscules in the root system, quantifying the presence of arbuscules in the whole root system (Trouvelot et al., 1986).

Grain yield and characteristics: At the time of maturity, yield data were recorded. For this purpose, plants of three random parcels of 15 m2 (10 x 1.5 m), not close to the margins of the field, were collected for each treatment. After a manual separation of seed from the cob, grain was weighed and a subset of 500 g was stored in oven at 105°C for moisture content analysis. Grain yield was then corrected for the standard

moisture (14%). The chemical analyses of grain were carried out on samples from the eight different fields (a subset from ten replicates/field). Moisture, crude protein, ether extract, ash, crude fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), non-structural carbohydrates (NSC), were performed according to AOAC methods (2002). NSC were calculated as a difference.

NIR and electronic nose analyses: In the present paper, radiometric analyses and olfactometry tests were carried out on 80 maize grain samples collected from the eight different fields (ten replicates/field) in the second cropping year. Radiometric tests were performed by an ADS Field Spec HH PRO (Analytical Spectral Devices, Boulder, USA) 2500 nm (Siesler, 2008). A probe (ASD A122300 highintensity reflectance hand-probe), served by external light source (2900 K color temperature quartz halogen light) to illuminate the object of interest, was used. The probe allows to collect reflectance spectra over an area 20 mm in diameter. For each sample, the spectrum was acquired directly from the Nallophan bag containing the maize grain. A panel covered by the Nallophan was used as white referencing. The spectra were collected with the Lab Spec Pro software 'sample spectrum count'. Olfactometric tests were performed by a ten-MOS electronic nose Air Sense PEN2 (AIRSENSE, Analytics GmbH, Schwerin, Germany). This system intercepts the aromatic and volatile molecules released from the matrix. The air flux method was used in this trial. Data were acquired using a needle inserted into the Nallophan bag containing the grain. The sample run lasted 20 sec followed by a 40 sec flushing. Each measurement was checked and acquired by Win Munster v.1.6 software.

Mycotoxin analyses: Mycotoxin analyses were carried out on samples from the eight different fields (ten replicates/field) of the second cropping year. Mycotoxins (fumonisin, aflatoxin B1, zearalenone, deoxynivalenol) were determined by ELISA test through the automatic system Chemwell (Awereness Technology, Inc., Palm City; USA). The kits used were Ridascreen ® Fumonisin (R 3401 R-Biopharm, Darmstadt, Germany); Ridascreen ® Aflatoxin B1 30/15 (R 1211 RBiopharm, Darmstadt, Germany); Ridascreen ® Zearaleon (R 1401 R-Biopharm, Darmstadt, Germany); Ridascreen ® DON (R 5906 R-Biopharm, Darmstadt, Germany).

Statistical analyses:

Root colonization: After having assessed normality by the Shapiro-Wilk test, differences between treatments were tested using the Kruskal-Wallis test within the NPAR1WAY procedure of SAS software (SAS 9.3 for Windows 2011), at statistical significance of 0.05.

Yield: Main effects (treatment, soil and irrigation) and their interactions were analyzed within the two years of experimentation and tested by F-test. The analysis of variance was performed using the GLM procedure of SAS software (2011).

Chemical characteristics: The effect of mycorrhization on chemical composition of grain was evaluated over the two different cropping years and mean were separated at 5% by tstudent test (GLM procedure of SAS 9.3 for Windows 2011).

NIR data: All the spectra, consisting of 2151 points in the Vis-NIR radiation, were first submitted to a mathematical pretreatment (Standard Normal Variate with Detrend, SNDV) and then the first derivative with gap 4 and smoothing 4 (1-4- 4) was calculated. The statistics used for the equation development and evaluation were the standard error of cross validation (SECV), and the coefficient of determination in cross validation (1-VR). Analysis was performed using WinISI II software, version 1.04, supplied by Infrasoft International (ISI, State College, PA, USA) according to Shenk and Westerhaus (1996).

E-nose data: The olfactometric measurement of the 10 MOS sensors registered during the first 20 sec were processed as contiguous vectors, into a kind of 200 points 'spectrum', also pre-treated with SNVD and 1-4-4. The same data analysis performed on NIR data was adopted.

RESULTS

Mycorrhizal root colonization: The results on mycorrhizal index are shown in Figure 1. The rate of mycorrhizal colonization was very high for both the treatments. F% and M% of the mycorrhizal colonization were higher in the maize inoculated respect to the control plants, 87.8 vs 80.3% and 26.8 vs 17.5%, respectively, (P<0,05). A% was also higher in inoculated plants, 9.7 vs 5.8%, (P<0,05). Assuming the effect of mycorrhizae were of interest in terms of hydro-geological severity, the comparison was performed under the most unfavorable condition (non-irrigated). PF soil was selected owing to its lighter and coarser texture than CB soil. Indeed, those characteristics are related to a lower water holding capacity. Arbuscules and vesicles observed in maize root fragments are shown in Figure 2. The arbuscules are highly branched hyphal structures filling the root cortical cells and represent the key structures of the symbiosis where most of nutrient exchange between fungi and plant occur (Sawers et al., 2008). The vesicles are lipid storage organs. The root system of experimental maize plants is shown in Figure 3a and 3b. The higher mycorrhizal colonization found in inoculated maize seems not to have clearly affected the development of the root system that are not different at glance.

Grain yield and chemical characteristics: Table 1 shows maize grain yield. Mycorrhizal treatment did not affect yield in terms of maize grain, but yield of maize cultivated on CB soil plots was lower than PF soil over both the years (P<0,05). No significant interaction was found between soil and mycorrhizal treatment. The effect of irrigation was not univocal, depending on the year. Therefore, in the first year no difference came out according to the water treatment while in the second yield was higher under wet conditions (P<0,01). Again, no meaningful interaction was found between irrigation and treatment and between soil and irrigation. Table 2 shows the chemical composition of maize grain. Mycorrhizal treatment did not affect chemical characteristics of maize grain except for lipid (P<0,01), ash (P<0,05), and part of the fibrous fractions (ADF, P<0,05) that resulted significantly higher in inoculated maize grain for the first cropping year. In both years NDF content, the highest fibrous component, tended to be lower in modulus in mycorrhizal maize grain, even though this difference was never statistically meaningful.

NIR and electronic nose: For NIRs data (Tab. 3), the mycorrhization factor gave a high coefficient of determination in cross validation (1-VR=0.86) whilst the influence of irrigation and soil factors were slightly lower (0.79). On the contrary the analysis of the e-nose data (Tab. 4) showed a strong influence of the soil (0.75) rather than mycorrhization (0.63) but the influence of irrigation was very low (0.33).

Mycotoxins: Preliminary analyses about the content of main mycotoxins in maize grain were performed (data not shown; Dr. Locatelli personal communication). The values of Fumonisin content did not change in mycorrhized samples when compared to controls. Aflatoxin B1, Deoxynivalenol (DON) and Zearalenone (ZEA) contents were close to the limits of detection in all samples, so comments about the content of these mycotoxins according to treatments cannot be rationally formulated.

DISCUSSION

Mycorrhizal root colonization: The commercial inoculum applied in the trial increased the AM colonization of maize roots although a considerable abundance of mycorrhizae in uninoculated plants was also found. As a matter of fact, mycorrhizal index (F%) values registered in uninoculated maize were very high (80.3%), when compared with related studies. The AM colonization of uninoculated maize produced under field conditions varied according to different Authors: 39.7% in maize as following crop after Trifolium alexandrinum (Pellegrino et al., 2011), 25.1-61.2% in two consecutive years (Sousa et al., 2012), 45% (Cheeke et al., 2013) and 55.3% (Berta et al., 2014). Autochthonous AM fungi can colonize roots of most plant species, including maize (Smith and Read, 2008), but it was clearly demonstrated that the soils exposed to intensive cultivation have extremely low number of indigenous AM fungal propagules (Li and Zhao, 2005; Subramanian et al., 2009). Despite intensive agricultural practices have been applied along many years in the farm where this experimental trial was carried out, the frequency of mycorrhizae found in uninoculated plants remained high. In general, intensive agricultural practices include monoculture, fertilizer, pesticide and herbicide

applications and conventional tillage. The latter, considered a disturbing practice, is supposed to reduce the function of AM symbiosis through the breakdown of their hyphal network in the soil (Jasper et al., 1989; Menendez et al., 2001). However, as results from meta-analysis of numerous studies (Lekberg and Koide, 2005), conducted to evaluate the effects of different agricultural practices on mycorrhizal colonization, the control of disturbance slightly contributes to increase mycorrhizal colonization. Disturbance is supposed to mostly affect hyphae (Evans and Miller, 1988; Evans and Miller, 1990), whereas spores and colonized root pieces may still result infective. The monoculture, as reported by Lekberg and Koide (2005), did not affect subsequent mycorrhizal colonization when compared with crop rotations including mycorrhizal plants. A further factor to consider for mycorrhizal colonization is the concentration of available P in the soil. The experimental plots were well supplied with P content (Raglione et al., 2011) Under such conditions, the colonization of root by AM fungi is not favored and is often suppressed (Jensen and Jakobsen, 1980; Al-Karaki and Clark, 1999; Kahiluoto et al., 2001). M fungi from fertilized soil produced fewer hyphae and arbuscules and consequently supplied their host with fewer inorganic nutrients from the soil (Johnson, 1993). Concerning the mycorrhizal colonization of inoculated plants of this trial, F (87.8%) was lower than the expected based on some similar available studies in field conditions (Berta et al., 2014). The significant, but not remarkable differences between the rates of root colonization in inoculated plants respect to uninoculated ones may be explicated considering the difficulty the AM inoculum has to face in soil colonization. In fact, the survival of the introduced microorganisms, as well as their activity on the host plant, are restricted by the competition with the resident microflora. (Hazarika et al., 1999; Zhang et al., 2011).

Root development, grain yield and chemical characteristics of maize: The root systems of inoculated and uninoculated maize showed a comparable size, as reported in the pictures (Fig. 3a and 3b). These results disagree with those of other Authors (Berta et al, 2014; Subramanian et al., 2014), who found a higher length and volume of inoculated maize roots. The MICOSAT F® inocula did not affect maize grain yield for the two years. Even though mycorrhizal frequency is not always a good predictor of plant productivity, results of metaanalysis (Lekberg and Koide, 2005) showed that the increase of mycorrhizal colonization is related with some benefits for plants. The lack of effect of inoculum on grain yield could be due to: a) small difference in mycorrhizal colonization between inoculated and uninoculated maize; b) high soil available P content; c) inefficiency of the markers used to evaluate the advantage of MICOSAT F® inoculum in specific soil and climate conditions. A recent study (Sabia et al., 2015) revealed a significant effect of AM seed inoculation on yield of forage maize cultivated within a low input system, including low P input. Regarding the different soils used in this trial, grain yield of PF resulted higher in both the years respect to that of CB soil. The characteristics of the CB soil, i.e. high clay content, compactness and tendency to surface fissuring, make it less suitable for maize cropping (Passioura et al., 1991). Regarding the irrigation, the different weather conditions over the two years may justify the results: no significant difference between dry and irrigated cropping in 2011 and significantly higher yield for irrigated crop in 2012. The period between June and August 2011 was characterized by abundant average rainfall (41.6 mm) and mild average temperature (24.1°C). In the same period of 2012 only 4.7 mm of rain fell and the average temperature was 25.9°C. The lack of irrigation effect on grain yield of maize cultivated in 2011 could have been due to the rainy and damp weather conditions over that cropping period. On the other hand, 2012 was much drier and although only an emergency sprinkling irrigation had been performed, the effect was evident. The absence of significant interaction between mycorrhizal colonization and soil and between mycorrhizal colonization and irrigation suggests that the used inoculum did not improve the maize productivity in soil with different texture and different attitude to drought. Concerning the grain chemical characteristics, a different response was obtained according to the year. In 2011 the higher ether extract, ADF and ash content registered in grain of mycorrhized maize is consistent with data presented by other Authors (Subramanian et al., 2014; Al-Karaki et al., 1998). We should stress a scarce impact of MICOSAT F and the major effect of the cropping year on maize production and quality. Perhaps, owing to the drier weather in 2012 when compared to 2011, the balance of the competition between plants and between plant and free-living microorganisms (Rennenberg

et al., 2009) may result modified, leading to a reduction of efficiency of MICOSAT F in promoting plant's absorption of specific minerals. Nevertheless, the NIRs analysis of grain of the second cropping year allowed to clearly separate between mycorrhized and not mycorrhized even though, in this case, no differences were highlighted by the chemical analysis of proximate composition. That last event confirms multifactor approaches, and particularly NIRs and multivariate analysis, as strong tool for discriminating complex matrices. As a matter of fact, NIRs, thanks to the detection of the overtones and combination bands that are specific for different functional groups, besides the fundamental transition from the ground to the excited state of the molecules (harmonic vibration model), can give a great deal of additional information. Therefore, NIR spectra are very powerful tool for predictive as well as discriminant analyses. As for the electronic nose, soil factor affected the volatile compound of maize more than mycorrhizal inoculum. An electronic nose is designed to recognize different species in the headspace and its selectivity is limited. The response is often subjected to noise, drift, and signals pertaining to other factors and pre- and post-treatments are required in order to minimize those effects (Sheebha and Subadra, 2014; AlMaskari et al., 2014; Hines et al., 1999). The stronger and/or confusing effects of other factors such as soil and water regimen may have veiled the effect of VAM inoculation. Since mychorrizae are supposed to change volatile compounds production in plants (Fontana et al., 2009; Leitner et al., 2010), differences could be isolated and appreciated under strictly controlled conditions at greenhouse or pots, where the only involved factor is inoculation.

Conclusions: The inoculum (MICOSAT F®) increased the AM colonization of maize roots even though it probably underwent to a strong competitive pressure by the autochthonic microflora that limited its performances and vitality. Indeed, a considerable number of mycorrhizae was detected in uninoculated plants as well. The NIRs indicated some physical differences between inoculated and uninoculated samples and resulted efficient for characterization of mycorrhized maize grain. On the other hand, e-nose's response is more affected by soil conditions than by mycorrhizal colonization. Further in-depth studies on AM fungi physiology, their function and the interactions with crops and environmental conditions are required to thoroughly explain these results. Compounds involved in the expression of inoculation's effect as well as each specific role must be characterized. Eventually, plants should be tested in different soils and conditions to clarify the effect of MICOSAT F® on maize yield and composition for food and feedstuff production.

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