Effect of transgenic soybean on functional groups of microorganisms in the rhizosphere in soil microcosm

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ABSTRACT

Neglected Glyphosate-resistant (GR) crops are common in agro-ecosystems mainly due to its benefits of weed management. However, the effect of GR crops on soil ecosystem and on non-target soil organisms need to be monitored. The effect of two transgenic soybeans GR on soil microorganisms, soil enzymes, microbial biomass and plant growth were evaluated. The experimental design was conducted as factorial arrangement with two GR soybean varieties, the Londrina (RR 59) and its near isogenic non-GM 59 Londrina called VAR 1; the second was Valiosa soybean (RR Conquista) and its near isogenic non-GM Conquista - Uberaba soybean called VAR 2. The plants were inoculated with arbuscular mycorrhiza fungi and rhizobia. The results showed that significant differences were observed among GM plants and their parental non-GM only for N biomass, AM colonization and cellulase activity. The presence of AM fungi had great influence on the functional groups of microorganisms while some enzymes activity decreased.

Key words: Arbuscular mycorrhiza, glomus, soybean, transgenic, genetic modified, risk analysis.

INTRODUCTION

The advancement of biotechnology has brought the possibility to insert genes from one organism into another cell, creating transgenic organism such as transgenic soybeans plants (Marinho et al., 2014). They received genes that produce some proteins, which protect them against a herbicide named glyphosate. This technology resulted in lower production costs, reducing the application of total pesticides, and decreasing environmental contamination (Park et al., 2011).

The glyphosate resistant transgenic soybean has been cultivated for more than twenty years and since 1996 represents the most prevalent transgenic crop (Nakatani et al., 2014; Duke et al., 2012; Bonny 2011). However, the effect of genetically engineered soybean on soil microcosm has not yet been completely clarified (Babujia et al., 2016; Guan et al., 2016). Soil microorganisms drive agro-ecosystem functions on nutrient turnover, soil structure. Transgenic crops can affect soil microorganisms due to differences in the amount and composition of root exudates (Liang et al., 2014), gene transference (Pontiroli et al., 2007), besides the effects caused by differences in management practices for transgenic crops, e.g., pesticide applications (Tsatsakis et al., 2017; Sessitsch et al., 2004), tillage and fertilizer application (Motavalli et al., 2004). These crops can also affect soil microorganisms due to differences in the amount and composition of decomposing crop residues (Lu et al., 2010; Icoz and Stotzky 2008).

Therefore, microbial functionality in natural ecosystem or agricultural soils is an important bioindicator of soil quality, as well as soil of microbial biomass (Kaschuk et al., 2010). The interaction of plant-microorganism is influenced by root exudates, which is the nutrient source to microbial community, including biological nitrogen fixation (BNF) and arbuscular mycorrhizal fungi (AM) (Andrade 2004).

Proteins from transgenic plants can be released and selectively influence the microbial community, stimulating or suppressing microorganism growth (Tsatsakis et al., 2017; Dunfield and Germida 2004). The question that remains is whether these new proteins can cause any effect on the function of soil microorganisms in tropical soils.

The aim of this study was to evaluate the influence of glyphosate resistant soybean inoculated or not with AM fungi on functional groups of soil microorganisms.

MATERIALS AND METHODS

Experimental design

The experiment was carried out using two different varieties of GM soybean glyphosate resistant and its near isogenic non-GM. The experimental was conducted in a completely randomized block design 4 x 2 x 1 x 5, (4) two GM soybean and non-GM near isogenic; (2) AM and non-AM plant and (1) one harvest 43 days after germination with (5) five replications (n = 40).

Plant growth and soil conditions

The soil from Londrina, PR in Brazil was used and is classified as a Rhodic Ferralsol (FAO 1994), medium texture, with the following chemical composition: Al 0.3 cmol; Ca 1.7 cmol; Mg 0.7 cmol; K 0.07 cmol; H + Al 4.9 cmol; C 10.4 g; P 2.2 mg, all in dm^{-3} of soil; pH 4.6. The substrate (soil sand 3:1 was steam-sterilized for 1 h on three consecutive days. In each pot of 500 g of substrate was added, and a microbial community, except for AMF, was restored with 10 mL of soil extract filtered. The soybean varieties were Londrina RR 59 and their near isogenic non-GM 59 Londrina called VAR 1, and the second was Valiosa soybean (RR Conquista) and its near isogenic non-GM Conquista – Uberaba soybean called VAR 2. Seeds were surface sterilized with hypochlorite solution (2%) for 2 minutes and rinsed 5 times with sterile distilled water. Six seeds were sowed in each pot, and one seedling was left four days after germination.

The seedlings were inoculated with two *Bradyrhizobium* strains, *B. japonicum* SEMIA 5080 and *B. elkanii* SEMIA 587, grown in YMA media (Vincent 1970) in a Petri dish at 28ºC for 7 days. Each strain was suspended in sterile saline and approximately 10^8 CFU mL^{-1} was inoculated around the seedling.

AM fungi inoculum of *Glomus clarum* (spores, mycelia, root fragments), was obtained from pots grown with *Brachiaria decumbens* kept in a greenhouse. Each pot received 2 g of AM fungi inoculum 1 day before seeds were sowed over the inoculum and the experiment was kept in greenhouse conditions (28 °C and 25 °C for day and night respectively, relative humidity 60%).

Functional groups of microorganisms assays

The colony forming units (CFU) were estimated by plate counts of aliquots from serial 10-fold dilutions, where 1 g rhizosphere soil (stuck to the roots) was collected from each plant and suspended in sterile saline (NaCl 0.85%). Aliquots of 50 µL of each respective dilution were spread on duplicate petri dishes with an appropriate culture media for enumerating the populations were used as following: 10^{-6} for heterotrophic bacteria (Ferreira et al., 2003), 10^{-4} for actinomycete (Küster and Williams 1964), fluorescent pseudomonas (Katoh and Itoh 1983), proteolytic (Wood 1980, as amended by Andrade 2004) and phosphate solubilizing (Sylvester-Bradley et al., 1982), 10^{-3} for amylolytic (Pontecorvo et al., 1953), cellulolytic (Wood 1980), and saprophytic fungi (Ferreira et al., 2003), flagellate and ciliate protozoa (Woomer 1994).

The soil samples were kept at 4 ° C before C and N biomass were determined. Soil microbial biomass of carbon (MBC) and nitrogen (MBN) were estimated by fumigation-extraction (Vance et al., 1987).

Mycorrhizal colonization

Percentage of root colonization by AM fungi was determined after staining root (Phillips and Hayman 1970) and estimated by gridline intersection method (Giovanetti and Mosse 1980).

Plants

Plants were harvested and the fresh weight of the roots, shoots, and nodules was recorded. To estimate the dry weight of roots, shoots and nodules were dried for 48 h at 55°C before recording. Root length was estimated according to the gridline intersection method (Newman 1966).

Enzyme activity

Before sampling, the soil moisture was determined by gravimetric estimation, incubating the soil at 105ºC 24 h^{-1} and weighed. To determine the enzyme activity, the soil samples were kept at 4 °C before the assays were carried out.

The enzymatic activity from biogeochemical cycling was also evaluated, from C cycling (dehydrogenase and cellulase), N cycling (urease and asparaginase) and P cycling (acid phosphatase).

The soil enzyme activities were determined as follows: Dehydrogenase activity (Casida et al., 1964), Cellulase activity (Schinner and Von Mersi 1990), Urease activity (Tabatabai and Bremner 1972), Asparaginase activity (Frankenberger and Tabatabai 1982), Acid phosphatase activity (Tabatabai and Bremner 1969).

Statistical analyses

The dataset was submitted to analysis of variance (one-way ANOVA) and mean comparisons by Tukey´s test (p<0.05). In addition, multivariate analyses were performed with Canoco 4.5 for Windows software. The Principal Component Analysis (PCA) was carried out in order to verify the relationships among variables in transgenic and non-transgenic plants.

RESULTS AND DISCUSSION

The insertion of genes which protect the plant against glyphosate did not affect the functional groups of microorganisms when compared with VAR 1 near isogenic plant non-GM, except for N microbial biomass that increased. Additionally, the presence of *G. clarum* showed great effect on almost all the microbial populations evaluated, increasing fluorescent pseudomonads, P solubilizing, proteolytic, ciliate and flagellate and decreasing cellulolytics (Table 1).

Table 1. Effect of transgenic soybean glyphosate resistant VAR 1 and AM fungi on functional groups of microorganisms and N biomass after 43 days of germination. AM colonization (AM), fluorescent pseudomonas (FP), P solubilizing (PS), cellulolytic (Cel), amilolytic (Ami), proteolytic (Prot), ciliate (Cil), flagellate (Fla), N microbial biomass (N biom).

Treatment	AM	FP	PS	Cel	Ami	Prot	Cil	Fla	N biom
	(%)	(log cfu g dry soil^{-1})					(log NMP)		(µg N g dry soil^{-1})
Plant (n= 60)									
Non-GMP	73.70a[1]	5.75a	5.52a	5.58a	5.88a	6.22a	3.95a	3.49a	17.77a
GMP	70.30a	6.00a	5.58a	5.58a	5.91a	6.50a	4.06a	3.28a	11.08b
AM Fungi									
Non-AM	-	5.54b	5.15b	5.84a	5.88a	5.94b	3.55b	2.93b	14.91a
AM	-	6.21a	5.95a	5.31b	5.92a	6.78a	4.46a	3.83a	13.94a
ANOVA (p values)									
Plant	0.583	0.140	0.783	0.992	0.682	0.165	0.697	0.592	0.022
MA	-	<0.01	<0.01	<0.01	0.632	<0.01	<0.01	0.023	0.735
PGM*MA	-	0.797	0.636	0.512	0.116	0.799	0.841	0.487	0.153

[1]Means with the same letter are not significantly different byTukey´s test *(p< 0.05)*.

VAR 2, the near isogenic non-GM improved AM colonization, and no differences on the functional groups of microorganisms between GM and non-GM plants in VAR 2 was observed. However, AM colonization increased fluorescent pseudomonads, P solubilizing and proteolytics and decreased cellulolytics as observed in VAR 1. The amylolytic population was decreased by AM fungi in VAR 2 (Table 2).

In the PCA correlation among functional groups of microorganisms' population and transgenic soybean VAR 1, the factorial plan considering the axes 2 and 3, which represent the variability, axis 2 explains 45.9% and axis 3 33.8% respectively. The inoculation with AM fungi provided a separation among treatments, regardless of transgenic. Thus, the non-AM plants showed a positive correlation with axis 2, while those inoculated were negatively correlated with the line. The population of proteolytic, amylolytic, fluorescent pseudomonads, heterotrophic bacteria, ciliates and flagellate had better correlation with AM plants in relation to axis 2. Axis 3 showed more correlation with cellulolytic in non-AM plants and P solubilizing positioned in the opposite of axis (Figure 1).

In the PCA correlation among functional groups of microorganisms and transgenic in VAR 2 (Figure 2), the factorial plan considered axes 2 and 3 with the highest variability representation. The variability percentage presented in axis 2 was 10.1% while in axis 3 it was 71.4%. In VAR 2, the AM fungi also provided a separation among treatments, regardless of transgeny. Thus, the AM plants showed a positive correlation with axis 3, while a non-AM plant was negatively correlated with that line. Fluorescent pseudomonads and P solubilizing showed greater correlation with AM plants, while cellulolytic and amylolytic had an opposite relation, and correlated with non-AM plants.

The microbial biomass is a reservoir of living nutrients in the soil, such as C, N and P, which are available by microbial exudates and by lysates after the death of a microbial cell, and can have been influenced by glyphosate resistant soybean. Indeed, previous work showed that GMs plants may cause changes on soil organisms and on the processes mediated by them, changing the root exudates and thus interfering in nodulation, mycorrhization and pathogen establishment (Bohm et al., 2009; Kremer and Means 2009; Wenke and Lianfeng 2008). In addition, genetic modified wheat changed the rhizosphere population by selectively stimulating the growth of specific organisms able to use novel proteins released from transgenic plants (Nakatani et al., 2014; Dunfield and Germida
2004). Similar effects were observed in canola (*Brassica nigra*) rhizosphere with glyphosate resistance genes, these changes were dependent on the cultivar used and not on the transgenic (Siciliano and Germida 1999). The same results were observed in soybean in this study, except for N biomass in VAR 1 and AM colonization in VAR 2. In contrast, changes caused by transgenic crops in soil biology did not differ qualitatively and quantitatively when compared with human activities or natural variations in the soil (Babujia et al., 2016; Givens et al., 2009; Nakatani et al., 2014; Ondreickova et al., 2014; Park et al., 2011).

Soil enzymes

In VAR 1, all the enzyme activities evaluated were not influenced by transgenic plants. However, AM inoculation influenced most of the enzyme activity except for urease, where no effect was observed. The AM inhibited the activity of asparaginase, dehydrogenase and phosphatase, and cellulase activity was improved by AM fungi (Table 3).

In VAR 2, transgenic plants did not show any effect on enzyme activity, except for the increase of cellulase activity. AM fungi showed different effects. Urease and cellulase activity were improved, and asparaginase activity highly decreased by AM inoculation. No effects were observed for dehydrogenase and acid phosphatase (Table 4).

Changes in microbial community composition should influence enzyme activity in the rhizosphere (Paz-Ferreiro and Fu 2016). The fact that cellulase showed greater activity in AM plants in both varieties may be related to the microbial activity increase due to the AM effect and competition by soluble C source, and the low amount of organic matter in soil, which increase enzyme activity to obtain nutrients. In general, high enzymatic activity can also be linked to a greater efficiency of organic mineralization products from the roots (Yang et al., 2008).

In PCA, the correlations among enzymatic activity and treatments in VAR 1, the percentage of variability explained in axis 1 was 71.5%, while in axis 2 it was 7.3%. The AM inoculation provided a separation among treatments, regardless of transgenic plants. In this case, considering axis 1, which best explains the variability, the non-AM plants showed a positive correlation with that line, while AM plants were negatively correlated with that line. Variables dehydrogenase and urease had better correlation with non-AM plants, while cellulase showed an opposite position and was correlated with AM plants (Figure 3).

Table 2. Effect of transgenic soybean glyphosate resistant VAR 2 and AM fungi on functional groups of microorganisms and N biomass after 43 days of germination. AM colonization (AM), fluorescent pseudomonas (FP), P solubilizing (PS), cellulolytic (Cel), amilolytic (Ami), proteolytic (Prot), ciliate (Cil), flagellate (Fla), N microbial biomass (N biom).

Treatment	AM	FP	PS	Cel	Ami	Prot	Cil	Fla	N biom
	(%)	log cfu g dry soil^{-1})					(log NMP)		(µg N g dry soil^{-1})
Plant (n= 60)									
Non-GMP	80.50a[1]	6.16a	5.39a	5.68a	5.99a	6.64a	4.13a	3.12a	12.48a
GMP	64.60b	6.13a	5.49a	5.69a	6.04a	6.59a	3.88a	2.97a	12.34a
AM Fungi									
Non-AM	-	5.84b	4.91b	6.01a	6.16a	6.47a	3.69b	2.75a	12.17a
AM	-	6.46a	5.97a	5.37b	5.87b	6.77a	4.32a	3.33a	12.66a
ANOVA (p values)									
Plant	0.077	0.854	0.626	0.912	0.687	0.781	0.327	0.708	0.876
MA	-	<0.01	<0.01	<0.01	<0.01	0.105	0.019	0.159	0.964
PGM*MA		0.270	0.249	0.679	0.187	0.597	0.509	0.300	0.854

[1]Means with the same letter are not significantly different byTukey´s test *(p< 0.05)*.

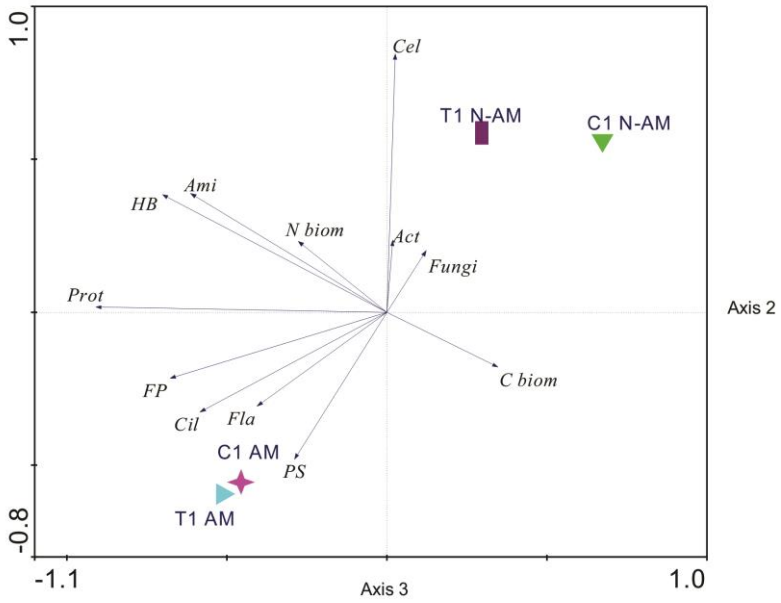

Figure 1. Factorial plan of Principal Components Analysis (PCA) based on functional groups of soil microorganisms in the rhizosphere of soybean VAR 1, 43 days after germination. [flagellate (Fla); ciliate (Cil); proteolytics (Prot); amilolytics (Ami); heterotrophic bacteria (HB); P solubilizing (PS); actinomycetes (Act); saprophytic fungi (Fungi); fluorescent pseudomonads (FP); cellulolytic (Cel); N biomass (N biom); C biomass (C biom)] and treatments [C1 N-AM (isogenic non-GM, non-AM, VAR 1); C1 AM (isogenic non-GM, AM, VAR 1); T1 N-AM: (GM, non-AM, VAR 1); T1 AM (GM, AM, VAR 1)]. Axis 1 = 45.9% and Axis 3 = 33.8%.

The PCA correlations among enzyme activity and treatments in VAR 2, the percentage of variability

explained in the axis 1 was 21.2%, while in axis 2 it was 65.7%. The inoculation with AM fungi provided a separation among treatments, regardless of transgenic, in axis 1 and axis 2. In this case, considering axis 2, that best explains the variability, AM plants showed a positive correlation with that line, while the non-AM plants were negatively correlated to that line. Asparaginase and acid phosphatase had a better correlation with non-AM plants, while cellulase and urease showed an opposite position and were correlated with AM plants. Note that axis 1, which explained a smaller percentage of variability, dehydrogenase and asparaginase were better correlated with non-AM plants, while the acid phosphatase showed an opposite position of these variables (Figure 4).

The urease activity did not differ in VAR 1, but in VAR 2. The urease and acid phosphatase activities were increased in *Retama sphaerocarpa* inoculated with *G. intrarradices* (Caravaca et al., 2003). Moreover, dehydrogenase, asparaginase and acid phosphatase activity were lower in AM plants and the presence of transgenic did not show any effect. The influence of AM fungi in the acid phosphatase activity is controversial, Caravaca et al. (2003) reported that AM plants showed high acid phosphatase activity in the rhizosphere. Otherwise, Joner and Jakobsen (1995) concluded that the acid phosphatase activity was not directly influenced by AM fungi, but by the change in exudates caused by AM-plant association.

Plant growth

The parameters related to the BNF showed no significant differences when compared GMs with non-GMs in VAR 1. However, the AM colonization increased nodules fresh and dry weight as well as nodules number, which showed higher differences when compared with non-AM plants. No differences were observed for transgenic plants in VAR 2, but AM fungi improved fresh and dry weight as well as nodules number.

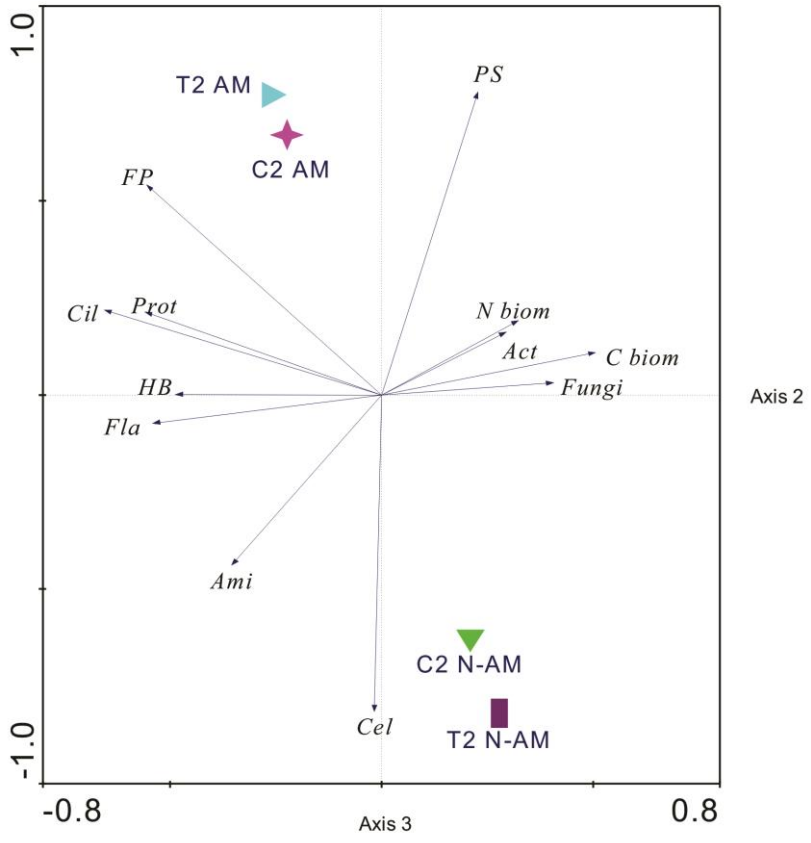

Figure 2. Factorial plan of Principal Components Analysis (PCA) based on functional groups of soil microorganisms in the rhizosphere of soybean VAR 2, 43 days after germination. [flagellate (Fla); ciliate (Cil); proteolytics (Prot) ; amilolytics (Ami); heterotrophic bacteria (HB); P solubilizing (PS); actinomycetes (Act); saprophytic fungi (Fungi); fluorescent pseudomonads (FP); cellulolytic (Cel); N biomass (N biom); C biomass (C biom)] and treatments [C2 N-AM (isogenic non-GM, non-AM, VAR 2); C2 AM (isogenic non-GM, AM, VAR 2); T2 N-AM: (GM, non-AM, VAR 2); T2 AM (GM, AM, VAR 2)]. Axis 2 = 10.1% and Axis 3 = 71.4%.

The transgenic did not affect plant growth, but AM inoculation increased shoot fresh and dry weight in

VAR 1.The same results were observed in VAR 2, except for shoot dry weight, where no differences were observed.

In the PCA, the correlation among plant growth and treatments in VAR 1, the percentage of variability explained in axis 1 was 67.8%, while in axis 2 it was 26.1%. The presence of AM fungi provided a separation between treatments, regardless of transgenic plants. The AM plants showed a positive correlation with axis 1, while the non-AM plants showed an opposite position. All variables showed better correlation with the AM fungi for axis 1. Analyzing axis 2, the total and specific root length were correlated better with on-AM plants (Figure 5).

In the ACP correlation among plant growth and treatments in VAR 2, the factorial plan was presented considering axis 2 and 3, which had the highest representation of variability. The percentage of variability explained in axis 2 was 34.6%, while in axis 3 it was 53.9%. In VAR 2, AM plants also provided a separation between treatments, regardless of transgenic plants. Thus, the AM plants showed a positive correlation with axis 3, while non-AM plants were negatively correlated with that line. The variables related to the shoot had a better correlation with AM plants, while the root fresh weight showed opposite position, correlating better with the non-AM plants, in relation to axis 3, that best explained the variability. Observing axis 2, the total and specific root length was correlated better with non-AM plants, while the variables related to the shoot had an opposite position (Figure 6).

Table 3. Effect of transgenic soybean glyphosate resistant VAR 1 and AM fungi on soil enzymes in the rhizosphere from C cycling [dehydrogenase (Dhd), cellulase (Ceu)]; N cycling [urease (Ure), asparaginase (Asp)], and P cycling [acid phosphatase (Aph)] 43 days after germination.

Treatment	Ure	Asp	Dhd	Aph	Ceu
	(µg N g dry soil^{-1})		(µgTFF g dry soil^{-1})	(µg p-nitrophenol g dry soil^{-1})	(µg glicose g dry soil^{-1})
Plant (n= 60)					
Non-GMP	39.40a[1]	20.50a	2.56a	190.94a	267.70a
GMP	39.90a	18.60a	1.90a	211.39a	278.93a
AM Fungi					
Non-AM	45.90a	25.80a	2.70a	214.12a	217.27b
AM	33.20a	13.30b	1.76b	188.21b	329.35a
ANOVA (p values)					
Plant	0.969	0.747	0.067	0.093	0.620
MA	0.277	0.043	<0.01	0.034	<0.01
PGM*MA	0.362	0.208	0.476	0.547	0.529

[1]Means with the same letter are not significantly different byTukey´s test *(p< 0.05)*.

It is widely known that AM fungi increases nodule formation in soybean as well as in shoot dry weight and N fixation (Meghvansi et al., 2008). Therefore, we may conclude that the presence of glyphosate resistance genes has low effect on the functional groups of microorganisms, where only a few parameters such as N-biomass in VAR 1 and AM colonization and cellulase activity in VAR 2 had significant differences. However, AM fungi had great influence on functional groups of microorganisms and soil enzyme activity in the rhizosphere of transgenic and non-transgenic soybean.

Table 4. Effect of transgenic soybean glyphosate resistant VAR 2 and AM fungi on soil enzymes in the

rhizosphere from C cycling [dehydrogenase (Dhd), cellulase (Ceu)]; N cycling [urease (Ure), asparaginase (Asp)], and P cycling [acid phosphatase (Aph)] at 43 days after germination.

Treatment	Ure	Asp	Dhd	Aph	Ceu
	(µg N g dry soil^{-1})		(µgTFF g dry soil^{-1})	(µg p-nitrophenol g dry soil^{-1})	(µg glicose g dry soil^{-1})
Plant (n= 60)					
Non-GMP	36.40a	15.80a	1.65a	189.99a	269.82b
GMP	45.70a	16.10a	1.66a	206.15a	342.82a
AM Fungi					
Non-AM	31.90b	24.90a	1.79a	202.01a	253.37b
AM	50.30a	7.00b	1.52a	203.14a	359.28a
ANOVA (p values)					
Plant	0.300	0.950	0.958	0.538	<0.01
MA	0.044	<0.01	0.331	0.922	<0.01
PGM*MA	0.617	0.854	0.209	0.974	0.272

[1]Means with the same letter are not significantly different byTukey´s test *(p< 0.05)*.

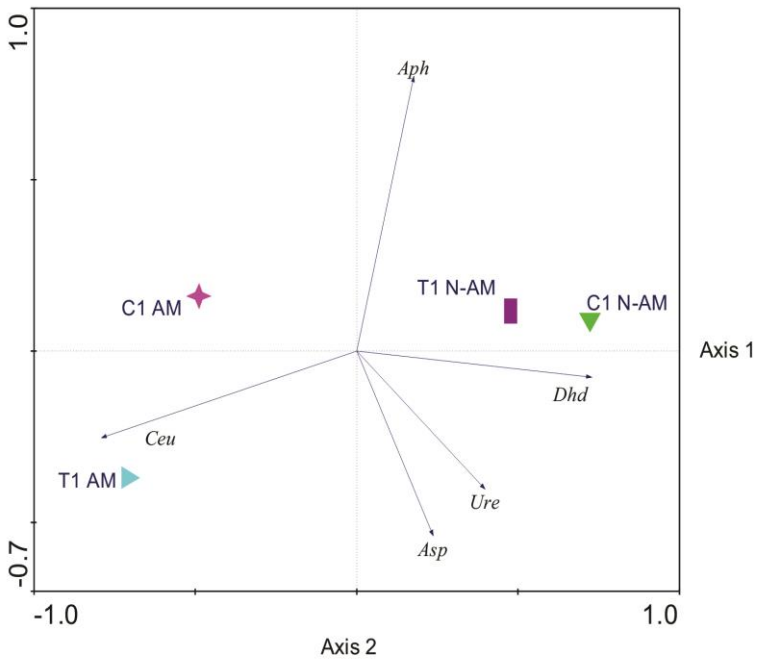

Figure 3. Factorial plan of Principal Components Analysis (PCA) based on soil enzyme activity in the rhizosphere of soybean VAR 1 43 days after germination, from C cycling [dehydrogenase (Dhd), cellulase (Ceu)]; N cycling [urease (Ure), asparaginase (Asp)]; P cycling [acid phosphatase (Aph)] and treatments [C1 N AM (isogenic non-GM, non-AM, VAR 1); C1 AM (isogenic non-GM, AM, VAR 1); T1 N-AM: (GM, non-AM, VAR 1); T1 AM (GM, AM, VAR 1)]. Axis 1 = 71.5% and axis 2 = 7.3%.

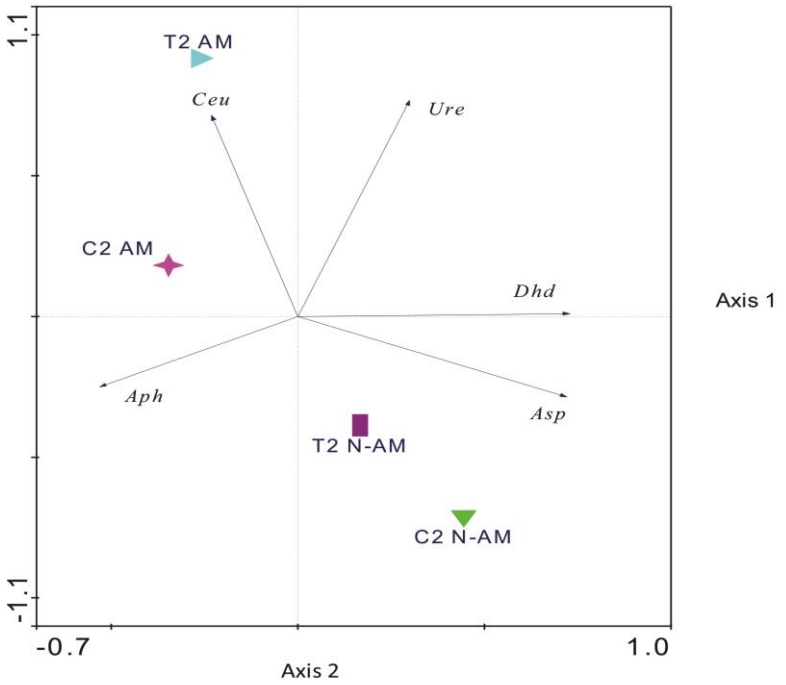

Figure 4. Factorial plan of Principal Components Analysis (PCA) based on soil enzymes activity in the rhizosphere of soybean VAR 2, 43 days after germination, from C cycling [dehydrogenase (Dhd), cellulase (Ceu)]; N cycling [urease (Ure), asparaginase (Asp)]; P cycling [acid phosphatase (Aph)] and treatments [C2 N-AM (isogenic non-GM, non-AM, VAR 2); C2 AM (isogenic non-GM, AM, VAR 2); T2 N-AM: (GM, non-AM, VAR 2); T2 AM (GM, AM, VAR 2)]. Axis 1 = 21.2% and axis 2 = 65.7%.

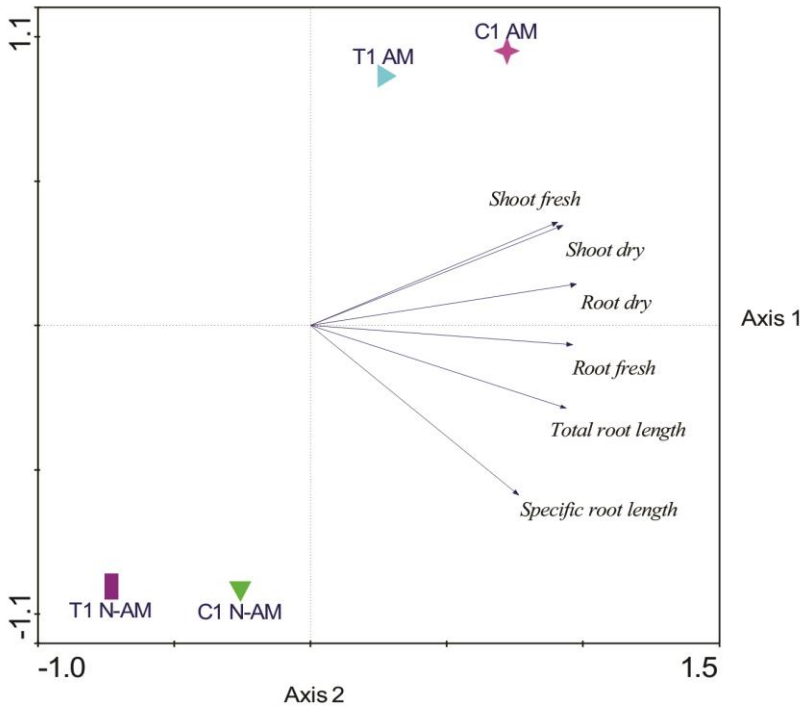

Figure 5. Factorial plan of Principal Components Analysis (PCA) based on plant growth parameters 43 days after germination [shoot fresh weight (Shoot fresh); shoot dry weight (Shoot dry); root fresh weight (Root fresh); root dry weight (Root dry); Total root length, Specific root length] and treatments [C1 N-AM (isogenic non-GM, non-AM, VAR 1); C1 AM (isogenic non-GM, AM, VAR 1); T1 N-AM: (GM, non-AM, VAR 1); T1 AM (GM, AM, VAR 1)]. Axis 1 = 67.8% and Axis 2 = 26.1%.

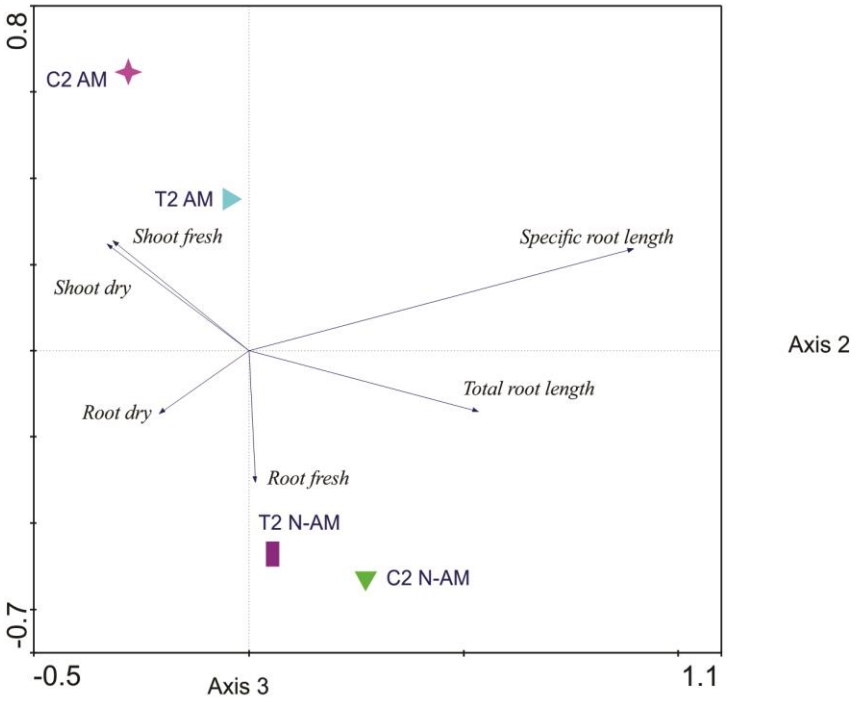

Figure 6. Factorial plan of Principal Components Analysis (PCA) based on plant growth parameters 43 days after germination [shoot fresh weight (Shoot fresh); shoot dry weight (Shoot dry); root fresh weight (Root fresh); root dry weight (Root dry); Total root length, Specific root length] and treatments [C2 N-AM (isogenic non-GM, non-AM, VAR 2); C2 AM (isogenic non-GM, AM, VAR 2); T2 N-AM: (GM, non-AM, VAR 2); T2 AM (GM, AM, VAR 2)]. Axis 2 = 34.6% and Axis 3 = 53.9%.

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